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Martin Willi Weber, Dr. med.

**Infection with the Respiratory Syncytial Virus in The
Gambia**

- Epidemiology, clinical spectrum, risk factors and sequelae -



A thesis submitted for the degree of Doctor of Philosophy to the Open
University in the field of Epidemiology

1998

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Table of abbreviations

A(L)RI	Acute (lower) respiratory tract infection
CCA	Chimpanzee coryza agent
CI	Confidence interval
CPE	Cytopathic effect
DNA	Desoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EMEM	Eagle's minimum essential medium
F (f)	Female
FBS	Foetal bovine (calf) serum
F protein	Fusion protein
G protein	Attachment glycoprotein
Hep-2	Human epithelial cell line 2
Hib	<i>Haemophilus influenzae</i> type b
IF	Immunofluorescence
Ig (A, E, G, M)	Immunoglobulin (A, E, G, M)
IRR	Incidence rate ratio
KD	Kilo Dalton
M (m)	Male
MRC	Medical Research Council
NCHS	National Center for Health Statistics, USA
NPA	Nasopharyngeal aspirate
n.s.	Not significant
OR	Odds ratio

P (p)	Probability
PIV	Parainfluenza virus
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcriptase polymerase chain reaction
RVH	Royal Victoria Hospital, Banjul
s.c.	Sub-cutaneous
SD	Standard deviation
TCID₅₀	Tissue culture infective dose 50
temp.	Temperature
vs.	Versus
WEC Mission	World-wide Evangelisation for Christ
WHO	World Health Organization

Publications

Some of the results of this thesis are reported in scientific papers which, at the time of submission of the thesis, have been published or are either in press or in the process of submission.

Weber, M.W., Mulholland, E.K., and Greenwood, B.M. Respiratory syncytial virus infection in tropical and developing countries. *Tropical Medicine & International Health* 1998; 3; 268-80.

Weber, M.W., Dackour, R., Usen, S., Schneider, G., Adegbola, R.A., Cane, P.A., Jaffar, S., Milligan, P., Greenwood, B.M., Whittle, H.C., and Mulholland, E.K. The clinical spectrum of RSV disease in The Gambia. *Pediatric Infectious Disease Journal* 1998;17:224-30.

Weber, M.W., Milligan, P., Hilton, S., Lahai, G., Whittle, H., Mulholland, E.K., and Greenwood, B.M. Risk factors for severe RSV infection leading to hospital admission in children in The Gambia. *International Journal of Epidemiology* in press

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Weber, M.W., Milligan, P., Sanneh, M., Awemoyi, A., Dakour, R., Schneider, G., Palmer, A., Jallow, M., Whittle, H., Mulholland, E.K., and Greenwood, B.M. The burden of RSV infection in The Gambia.

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I wish to dedicate this work to my family, my wife Barbara Wienkamp-Weber, our daughter Thordis and our son Jakob.

Abstract

The importance of respiratory syncytial virus (RSV) as a pathogen for acute lower respiratory tract infections in young children has been evaluated in a series of studies undertaken in The Gambia, West Africa. During the years 1993 to 1996, RSV occurred in yearly outbreaks in the rainy season. Mainly young children were affected, but the age distribution varied between years (median ages 3, 7, 8, and 5 months in the 4 years respectively). The estimated annual incidence of RSV infection leading to admission to hospital was 2.3% for children under 1 year of age; the proportion of all ALRI admissions due to RSV 19%.

In a case control study of potential risk factors for severe RSV infection, it was found that cases came from larger or more crowded households than controls; risk was particularly associated with larger numbers of children in the age group 3-5 years living in the extended household. Cases more frequently had a sibling who had died than controls. Controls were more likely to have been exposed to smoke from cooking fires than cases. Other protective factors were father's nationality and profession. Vegetables were included in the diet of controls more frequently than in the diet of cases. The number of asthmatic mothers was small in both groups.

In a prospective cohort study which compared RSV-ALRI cases with 2 control cohorts, it was found that the incidence of pneumonia was approximately 3½ times higher in cases than in controls. For wheezing, the rate was 7 times higher, but wheezing was less common than pneumonia. At 3 years of age, pneumonia and wheezing were uncommon in all groups. Incidence rates of ALRI were approximately twofold higher in the wet season than in the dry season.

In conclusion, RSV is an important cause of ALRI leading to hospital admission in the Gambia. Acute and subsequent morbidity is considerable, so prevention by vaccination would be worthwhile.

Chapter 1. Introduction

Acute respiratory infections are the main killer of children in developing countries, accounting for 4 million deaths in children under 5 years of age each year.^{81,179,180} Diarrhoeal illness, malaria in tropical regions and measles are the next most important infections. Malnutrition contributes to the mortality of all these diseases.^{179,180} Almost all acute respiratory deaths are due to lower respiratory tract infections such as pneumonia and bronchiolitis.^{29,52} The main causative bacterial pathogens of pneumonia are *Streptococcus pneumoniae* and *Haemophilus influenzae*.²²¹ Viruses have been identified as important causes of acute lower respiratory tract infection (ALRI) in several studies, but their contribution to mortality remains controversial.²⁹ Two main approaches to the management of ALRI in developing countries have been developed: prevention through vaccination, and case management with appropriate antibiotics and supportive therapy.²⁶⁵⁻²⁶⁷ Vaccines for the prevention of severe ARI which are available through the Expanded Programme of Immunisations are measles (which causes respiratory problems as part of the viral infection and through the subsequent immunosuppression which predisposes to acute bacterial infection) and pertussis vaccines.¹⁴ A vaccine against *H. influenzae* type b was found effective in The Gambia in the prevention of pneumonia, but its cost is high.¹⁷⁶ Unconjugated vaccines against *S. pneumoniae* are effective in older children but are not used widely,^{37,147} whereas conjugated vaccines are currently under development and need evaluation in developing countries.¹⁷⁶ Case management of ARI depends on the early identification of cases by peripheral health workers with limited training. It is based on the recognition of simple signs such as

respiratory rate and lower chest wall indrawing and the prescription of oral antibiotics for less severe cases and referral of more severe cases for inpatient treatment.²⁶⁶⁻²⁶⁸

Several studies have addressed the aetiology of ARI in the Gambia over the past 15 years.^{1,23,75-77,176,187,188,254} Early studies identified the causes of ARI in children who presented to hospital and outpatient departments;^{76,77,254} *S. pneumoniae* and *H. influenzae* were identified as the main bacterial causes. Later studies looked at aetiology in the important subgroups of malnourished children and infants under 3 months of age.^{1,174} More recently, information has been collected about the bacterial aetiology of ARI as part of the preparations for a vaccine trial with a conjugate vaccine against *S. pneumoniae* in the Upper River Division of the Gambia,^{187,188} and during the course of a trial of a conjugate vaccine against *H. influenzae* type b.^{176,249} Results of these studies are summarised in Table 4 in the section "RSV in The Gambia".

Early studies by Forgie *et al.* and the studies on the aetiology of ALRI in young infants and malnourished Gambian children suggested the importance of RSV. However, these studies were either short in duration or restricted to subgroups of children. Thus, it was considered important to perform a longer, more comprehensive study of the epidemiology of RSV infection in Gambian children to obtain information about seasonality, age distribution and clinical presentation. These data are essential to the planning of a future vaccine trial. Identification of cases of RSV infection during the course of the epidemiological

studies provided an opportunity to investigate potential risk factors for the infection and to evaluate its long term sequelae. Thus, the series of studies described in this thesis had the objectives outlined below.

1.1 Study objectives

- Description of the epidemiology of RSV infection in Gambian children based on surveillance of hospital cases of ALRI.
- Description of the community rate of infection with RSV in young children during outbreaks through visits to the compounds of index cases and controls.
- Description of the clinical spectrum of RSV infection among cases admitted to hospital, including the incidence of bacterial co-infection and outcome.
- Determination of the viral subtypes responsible for outbreaks, their relationship to viral strains found elsewhere, and their correlation with clinical severity.
- Identification of social and environmental risk factors for severe RSV infection.
- Determination of the frequency of subsequent respiratory infections in children who had been admitted to hospital with a severe RSV infection.

Chapter 2. A review of RSV infection in industrialised and developing countries

2.1 The virus

2.1.1 History

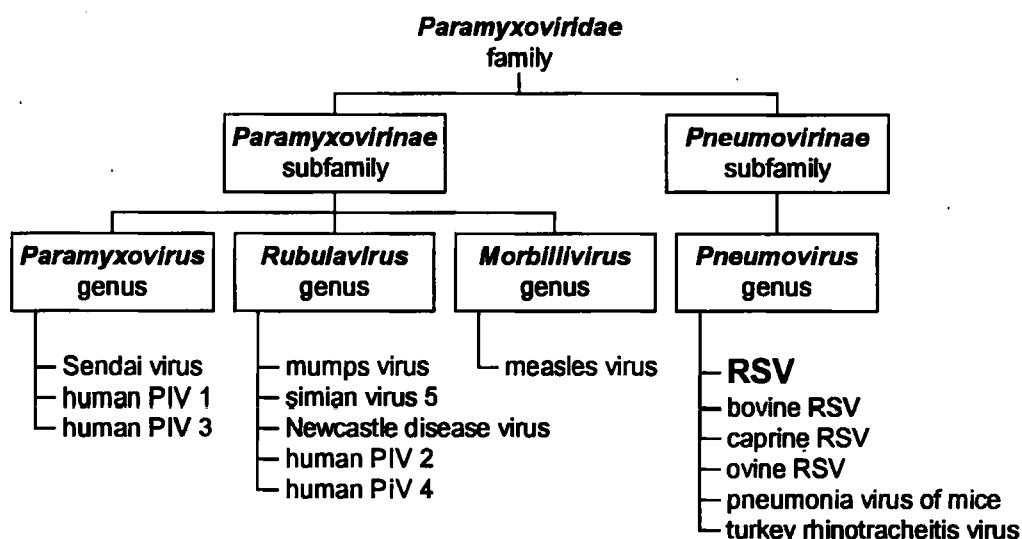
RSV was identified first in 1956 in a group of chimpanzees and accordingly called chimpanzee coryza agent (CCA).¹⁶⁸ It was speculated at that time that the chimpanzees were probably infected from a laboratory worker. Soon after, two agents indistinguishable from CCA were recovered from infants with bronchopneumonia,^{46,48} and the common virus of all three strains renamed “Respiratory Syncytial Virus” (RSV).⁴⁶ Over the next four years, studies in the USA identified RSV as the most important cause of lower respiratory tract infection in children and the main cause of bronchiolitis.^{24,47} Further studies from all continents found RSV or serum antibodies against RSV wherever these were sought.^{28,39,65,72,91,123,166,177,183,200,219,220,240}

2.1.2 Classification

Respiratory syncytial virus (RSV) is a medium sized enveloped RNA virus.^{20,128} It is classified in the family of viruses called *Paramyxoviridae*, and in the genus *Pneumovirus*.^{53,203} Bovine respiratory syncytial virus, ovine respiratory syncytial virus, caprine respiratory syncytial virus, pneumonia virus of mice, and turkey rhinotracheitis virus also belong to this genus (Figure 1).⁵³

Figure 1. Relationship of viruses in the family *Paramyxoviridae*.

Adapted from Collins et al.⁵³.



Note: Caprine and ovine RSV are possibly only subgroups of bovine RSV rather than distinct viruses.⁶ PIV: Parainfluenza virus

The genome of RSV contains genes for 10 main proteins.⁵³ The two main surface proteins which are important for the generation of an immune response are the fusion protein (F protein, 70 KD) and the attachment glycoprotein (G protein, 90 KD). Two groups of RSV strains have been identified using monoclonal antibodies, which are called group A and group B.^{9,84,173} They differ predominantly in the G protein; the F protein is well conserved between groups.¹²⁵⁻¹²⁷

2.1.3 Characteristics

RSV is a relatively labile virus. At 4°C, only 1 percent of infectivity remains after 1 week.¹⁰⁶ The virus withstands freezing and thawing poorly. In culture, RSV grows best in human diploid cell lines such as Hep-2 or HeLa cells.^{8,53,148}

The characteristic cytopathic effect of RSV in cell cultures is syncytia formation, from which the virus derives its name.¹³⁰

RSV is predominantly a human virus. RSV has also been recovered from chimpanzees, cattle, goats, and sheep, but human RSV grows less well in ruminant cells than bovine RSV.¹⁵⁵ Antibodies to RSV have been demonstrated in cats and dogs, however the meaning of this is unclear.²¹⁴

2.2 RSV infection in the industrialised world

2.2.1 Epidemiology

RSV infection is strongly seasonal in temperate climates. Outbreaks occur mainly during the winter months, extending into spring, and occur regularly every year.^{24,56,87,91,134,151,156,167,172,197,212,242} Outbreaks are usually sharp in onset and last between 2 and 5 months. In some settings outbreaks start early in one winter and late in the next winter.^{258,269}

The peak incidence of hospitalised cases of RSV is in the age group 2 to 5 months.^{11,86,197} Serological studies suggest that about half of all children are infected during the first year of exposure, and that almost all have been infected after the second outbreak that they encountered.^{33,134,226} In Washington, DC, 40% of all RSV infections in the first year of life involved the lower respiratory tract, and 1% of infected infants required admission to hospital.^{33,134} In Houston, Texas, 33% of the children with an RSV infection had a lower respiratory tract infection, and 1.6% required hospitalisation.⁸⁹ Males generally predominate at a

ratio of 1.5-2:1 among children admitted to hospital, ^{62,143,197} but the distribution between sexes is more equal in milder cases. ^{62,85,110,270}

Re-infection with RSV is common. A study in Tecumseh, Michigan, found 20% of 5-9 year old children to be (re-)infected within a year. ¹⁶⁷ The rate fell to 10 % in children 15-19 years old, and to 3 to 6% in adults between 20 and 50 years of age. RSV spreads effectively within families. ^{89,100} The infection appears to be introduced by children of school age. Bigger families have a higher rate of infection. The incubation period of illness from RSV has been reported as being between 2 and 8 days, most commonly between 4 and 6 days. ^{80,233,234}

2.2.1.1 RSV infection in elderly people

Recently, RSV has been recognised as an important pathogen in elderly people. Outbreaks have been described in nursing homes, affecting a high percentage of residents, with complications occurring in up to 15% of the infected persons. ^{4,94,107} Epidemiological studies correlating RSV outbreaks with excess deaths from respiratory infections indicate that RSV might be as important a cause of increased mortality in elderly people as influenza. ^{71,73,74}

2.2.1.2 Factors associated with RSV disease and protection

The following table summarises the dynamics of infection with RSV and the factors associated with transmission of the virus and protection as described in the literature.

Table 1: Factors associated with transmission of and protection against RSV.

Factor	Findings and comment	Reference
<u>Virus</u>		
Source	Human. Isolated from ruminants, chimpanzees, but animals not believed to be important for transmission.	Hall 95
Transmission and viability of the virus	Large droplets, small droplets inactivated quickly. Maximal stability at 60% humidity, less stable at 30% and 80% humidity.	Rechsteiner 208,209
Viability of the virus	RSV in freshly obtained infant secretions was recovered from countertops for up to 6 hr, from rubber gloves for up to 1 1/2 hr, from cloth gowns and paper tissue for 30-45 min, and from skin for up to 20 min.	Hall 99
Transmission distance	No infection in volunteers more than 6 feet away from infected infants.	Hall 96
Incubation period	4-6 days most common. (2-8 days as range)	Ditchburn 64 Stern 234
	Average 5 days in adult volunteers.	Kravetz 141
Shedding of virus	The virus was shed for prolonged periods. For the first seven days of hospitalisation, 92%-100% of the infants tested continued to shed virus. At discharge 87% were still shedding the virus.	Hall 97
	The mean duration of shedding for 23 patients until they were virus negative was 6.7 days with a range of 1 to 21 days.	Hall 98
<u>Immunity</u>		
Neonatal: maternal antibody	Earlier infection in infants with low maternal antibody levels.	Glezen 88
	The risk of reinfection was inversely related to the level of neutralizing antibodies in the serum.	Glezen 89
	Mean titre of maternal IgG antibody to RSV was significantly higher (P less than 0.001) in mothers whose babies remained uninfected than in those whose babies had proven RSV infection before 6 months of age.	Ogilvie 190
	Sera from a group of mothers whose babies escaped RSV infection during a local epidemic showed increased antibody levels to VPN41 when compared to sera from mothers whose babies had become infected with RSV within the first 6 months of life. In infants who remained uninfected with RSV during the first 12 months of life the maternal gift of antibody decayed to about 50% at 3 months with traces of antibodies detected in a few sera at 12 months. The antibody levels detected in the sera of infants less than 3 months old convalescent from primary RSV infection did not exceed the mean levels present in the serum of uninfected babies.	Ward 257
Breast milk	Eight out of 115 infants admitted to hospital with RSV infection had been breast-fed compared with 46 out of 167 controls.	Downham 68
IgA in breast milk	IgA antiviral antibody persisted in the milk of only four of 18 mothers. Similarly, antiviral IgG and IgM antibodies were not generally detected after the first post-partum week.	Toms 245

Table 1 ct.

Factor	Findings and comment	Reference
	Neutralizing activity against RSV was measured in milk samples from 17 healthy women whose infants had an acute infection with RSV and from 27 women with healthy infants. All milk samples were obtained 2-8 months post partum. Neutralizing activity was detected in 36 samples. No major difference in neutralising titres was observed between the two groups, and the titres were low.	Laegreid 144
Infection- and reinfection rates		
Infection- and reinfection rates	The infection rate was 68.8/100 children less than 12 months of age and 82.6/100 during the second year of life. Virtually all children had been infected at least once by 24 months of age, and about one half had experienced two infections.	Glezen 89
Frequency of reinfection	During epidemics the attack rate for first infection was 98 per cent. The rate for second infections (75 per cent) was modestly reduced ($P < 0.001$); that for third infections was 65 per cent. Amelioration of illness in subsequent attacks.	Henderson 111
Re-infection by age group	5-9 years of age: 19.7% 10-14 years: 16.9% 15-19 years: 10.1% adults: 3-6% re-infection per year.	Monto 167
Re-infection of adults	15 adults with previous natural RSV infection were challenged with RSV of the same strain group (A) at 2, 4, 8, 14, 20, and 26 months after natural infection. By 2 months about one-half and by 8 months two-thirds of the subjects became reinfected. Within 26 months 73% had two or more and 47% had three or more infections. The duration of immunity tended to increase after two closely spaced infections.	Hall 103
Infection within families	The virus infected 44.4 per cent of families, and 21.9 per cent of all members. All age groups had appreciable attack rates (with a range of 16.8 per cent in adults to 29.4 per cent in infants). In infected families, 45.9 per cent of members became infected, including 10 of 16 infants. Secondary attack rate for all ages was 27 per cent, and that for infants 45.4 per cent. An infant's older sibling appeared most likely to introduce the virus into the family.	Hall 100
Subtypes		
Cross-protection	Infection with subgroup A strains of respiratory syncytial virus provided some protection from a second infection with the homologous, but not the heterologous, subgroup of the virus. Primary group A infection elicited antibodies cross-reactive with group B virus in the PRNB and the ELISAS for GB and FB. In contrast, primary group B infection induced significant increases in mean concentrations of antibody cross-reactive with group A virus only in the FA ELISA. Second RSV infections caused by group B viruses in children with histories of primary group A infection induced heterologous rises in the PRNA and GA assays, suggesting that prior group A infection had primed for a more extensive cross-reacting antibody response at the time of second RSV infections with group B viruses.	Mufson 170 Muelenaer 169
Circulation	Three patterns of yearly outbreaks existed in 15 sequential years: (1) strong predominance of group A strains (9 years with 83%-100% A strains), (2) relatively equal proportions of group A and B strains (4 years), and (3) strong predominance of group B strains (78%-85%) in 2 years, separated by a decade. The first pattern of highly dominant A strains occurred in cycles of 1 or 2 consecutive years with a single intervening year in which B strains were greater than or equal to 40% of the isolates.	Hall 104

Table 1 ct.

Factor	Findings and comment	Reference
<u>Severity of disease</u>		
Lower respiratory infections	Although lower respiratory tract disease (LRD) was common (22.4/100 during year 1 and 13.0/100 during year 2), most children had only one LRD illness. Reinfection illnesses were generally mild, and risk of reinfection decreased to only 33.3/100 during year 4. Hospitalisation rate 1.6%.	Glezen 89
	West Virginia: 3.3 % hospitalised.	Belshe 26
	Immunity induced by a single infection had no demonstrable effect on illness associated with reinfection one year later, however, a considerable reduction in severity occurred with the third infection.	Henderson 111
	First year of life: 40% ALRI, 1% hospitalised.	Brandt 33
	The maximum yearly admission rate occurred among infants aged 1 to 3 months: 24.5 per 1000 of that age group were admitted to hospital. (industrial areas double the rate of rural).	Anonymous 11
Sex distribution	North East England: 2% of under 1 year olds admitted.	Martin 153
	1.8:1 male: female for LRI.	Glezen 85,86
	1.5:1 male : female for LRI.	Kim 134
	Equal for mild infection.	Glezen 85,86
<u>Risk factors</u>		
Crowding	Between 1 and 3 months of age, in multivariate analysis, only sex and the number of others sharing the room remained as significant direct risk factors. Being in day care was a significant risk factor in the 7- to 9-month age range.	Holberg 117
Socio-economic class	Children living in electoral wards in the two more deprived groups were more than 1.5 times as likely to be admitted (OR 1.67, 95%CI 1.25-2.24).	Spencer 231
<u>Seasonality</u>		
Winter	In temperate climates.	Hall 95
Rainy season	In most tropical climates with seasonal rain fall.	
Fluctuations between early and late outbreaks in different years	Early alternating with late, distance between peaks 15, 7, 14, 11, 13, 12, 14, 9 months.	Glezen 86
	March/April 1979 then January 1980 (West Virginia).	Belshe 26
	Over 10 years alternating early/late outbreaks, Turku, Finland.	Waris 258
	Outbreak December followed by outbreak March (16 months).	Wilcox 269
Missed outbreaks	One year in Gambia.	Forge 75
	29 months, Brazil.	de Arruda 59
	Three years in sequence with very little activity (1972-74), Chapel Hill, N.C. USA (?insensitive cell lines).	Henderson 103
	1984/85 Huntington West Virginia USA.	Mufson 171

2.2.2 Clinical features including long term complications

The first infection of an infant with RSV is almost always apparent, but clinical features vary between a runny nose and severe pneumonia.^{47,134} Children who develop a lower respiratory illness may do so again in the following years, but these episodes are generally less severe.^{89,100} The most common manifestation of RSV-ALRI is pneumonia, the ratio between cases of pneumonia and bronchiolitis ranges between 7:1 and 1:1.⁸⁵ Often the signs of both entities overlap, and pneumonia appears to be a continuum of bronchiolitis.⁹⁵

Pathological changes in the lungs of children who have died of RSV bronchiolitis include a peri-bronchiolar mononuclear infiltration, necrosis of the epithelium of the small airways, plugging of the lumina of the small airways, and hyperinflation and atelectasis.⁵ The most common signs of RSV-ALRI are cough (97-100%), rhinitis (56-82%), dyspnoea (50-78%), rhonchi (59-78%), wheeze (45-76%) and crepitations (27-72%)⁹⁵ Fever is less common in younger children than in older ones.²⁷ Clinical assessment of children is directed mainly at the detection of hypoxaemia. This is probably due to a low ventilation-perfusion ratio rather than to shunting through unventilated lung.^{101,160,261} Most children admitted to hospital improve sufficiently within 4 to 7 days to be discharged, but inflammation in the lung may persist longer with abnormalities in gas exchange and wheezing.^{101,105}

A few children require intensive care treatment with intubation, most commonly those with underlying illnesses such as lung disease of prematurity or congenital heart disease.^{143,150,182,235,255} The mortality from RSV is low, approximately 1-3% of hospital admissions die, mostly those with an underlying illness.^{11,226}

2.2.2.1 Further lung disease

RSV has been implicated in the development of chronic lung disease in subsequent years. Children with bronchiolitis have a higher incidence of further wheezing.^{145,164,202,204,225,227,263,264} Recurrent respiratory problems have been noted in up to half of the children, but seem to diminish as children become older. The role of atopy in this sequence of events is unclear, some studies show an increased risk of further wheezing in children of atopic parents,¹⁴⁵ whilst other studies have failed to do so.²²⁷

2.2.3 Treatment

Treatment of RSV infection is largely supportive, aimed at mechanically clearing secretions obstructing the airways and maintaining nutritional and fluid status and oxygenation.^{95,101} Children who are hypoxaemic receive supplemental oxygen. As the main abnormality in the affected lungs is a mismatch of ventilation and perfusion, relatively low concentrations of inspired oxygen are usually sufficient.¹⁰¹ This can be achieved with nasal prongs or nasal cannulae and low oxygen flow rates.²⁶⁰ Bronchodilators play a limited role in the treatment of RSV bronchiolitis.^{58,149,243,256} They appear to be least useful in younger children. Some authorities recommend a trial of bronchodilator therapy and to continue with this treatment if there is clinical improvement after the application.^{95,216} Steroids do not appear to be useful.¹³⁷ Specific antiviral treatment with ribavirin may be beneficial in high risk children, but its cost-benefit ratio is low.^{12,15,61,165,275} Secondary bacterial infections are uncommon, and children who received antibiotics had a higher rate of subsequent bacterial

infection.^{102,140,244} Therefore, routine antibiotic treatment of children with RSV infection is not recommended in developed countries.

2.2.4 Prevention

Prevention of RSV infection through vaccination has been hampered by a disastrous experience with a formalin inactivated vaccine in the 1960s which enhanced disease in vaccinated children and led to increased morbidity and also mortality.^{10,51,79,135} Further efforts to develop a vaccine were aimed first at understanding the mechanism of immune enhancement, but even today this is not completely understood.^{136,158,178,201} Development of a live attenuated vaccine was thus considered a safer approach, but the vaccine candidates were either over-attenuated and did not result in protection, or still caused lower respiratory infection.^{25,36,159,198,271,272} Another approach has been the development of vaccines based on purified subunits of the virus such as the F protein or a chimeric FG protein.^{72,177,200,220} There are some promising candidates, but currently, no vaccine is available for use in children.

Immunoglobulin with a high titre of anti-RSV antibody is effective at preventing severe RSV infection in children at high risk but its cost prevents more widespread use.^{92,93,161,162}

2.3 *RSV in the developing world*

2.3.1 Importance of RSV compared to other respiratory viruses

Earlier studies on the role of RSV in the aetiology of ARI used serology and/or viral culture. Immunofluorescence became available from the early 1980s onwards, and almost doubled the number of RSV positive cases.

2.3.1.1 Community Studies

Several longitudinal community studies of children have identified viruses as the cause of 11%⁷⁵ to 36%¹⁶⁶ of episodes of ARI. Five studies were performed without the use of immunofluorescence,^{32,138,166,196,239} and five with immunofluorescence.^{75,119,238,247,250} Whereas the overall isolation rate of viruses remained similar (average 21% versus 23%), the percentage of RSV identifications doubled from 23% to 44% (range 6%- 73%) in the studies that used immunofluorescence. As the overall isolation rate for viruses remained the same, viruses other than RSV were reported less frequently in studies which employed immunofluorescence. One study did not identify RSV activity over a period of 29 months despite apparently adequate methodology including antigen detection, immunofluorescence, and viral culture.⁵⁹

Table 2: Aetiology of ALRI in community-based studies in developing countries

Country & reference	study year	method	number studied	number virus positive	% virus positive	RSV	PIV (percentage of positive viral identifications)	Influenza	Adeno
Brazil 238	1987-89	IF & culture	50	15	30%	73%	7%	13%	0%
Uruguay 119	1985-87	culture & IF	858	131	15%	68%	8%	5%	8%
Philippines 247	1985-87	IF & culture	311	106	34%	38%	15%	7%	10%
Thailand 250	1986-87	IF & culture	674	109	16%	32%	34%	13%	15%
Gambia 75	1988-89	culture, IF & serology	491	55	11%	7%	25%	25%	33%
Panama 166	1963-64	serology & culture	150	54	36%	43%	46%		11%
Brazil 239	1980	culture	371	76	20%	3%	7%	11%	29%
Colombia 32	1986-88	culture & serology	340	108	32%	62%	22%	9%	5%
India, Bengal 138	1964-66	culture	4171	483	12%	2%	11%		33%
India, Bengal 196	1966-67	culture	1716	224	13%	5%	8%	3%	54%

2.3.1.2 Hospital in- and outpatient studies:

In studies performed in hospital outpatient departments or on patients admitted to hospital wards, a virus was found in 9%²¹¹ to 64%²¹⁷ of cases of ARI (average 41%). RSV was found in 4%⁴² to 96%²³⁸ of cases with a proven viral aetiology (average 39%). RSV was the leading cause of viral ARI, followed by influenza viruses, parainfluenza viruses and adenovirus.

Studies based on culture and /or serology only, which were all undertaken before the early 1980s,^{30,66,70,123,129,193,211,222,229,232,241,274} found on average a virus in 28% of cases. RSV accounted for an average of 32% (range 6-72%) of the samples positive for viruses. Studies which used immunofluorescence before 1985 did not change these proportions considerably: a viral aetiology was found in 56% of cases, with identification of RSV in 24% of culture positive cases (range 11 - 33%).^{67,109,199,207,217,253} From 1985 onwards, immunofluorescence was used either alone or in combination with serology or culture in 18 studies from developing countries.^{1,42,50,76,77,82,118,120,124,163,184,205,206,236-238,248,262} The overall viral aetiology rate did not change much from that found in earlier studies, with 33% of cases being attributed to a virus. However, RSV was implicated in 65% (range 27 - 96%) of the samples positive for a virus. Thus RSV was, on average, responsible for 17% of acute respiratory infections in children admitted to hospital in the developing countries where the studies were carried out.

Measles played a major role in some earlier studies on ARI done in Colombia,⁷⁰ the Philippines,²¹⁷ Papua New Guinea¹⁹⁹ and Kenya.^{109,253} However, by the mid 1980's, measles virus was no longer a significant cause of paediatric ARI in the studies reported, probably as a result of higher levels of immunisation.

Table 3: Aetiology of ALRI in hospital-based studies in developing countries

Country & reference	study year	method	number studied	number virus positive	% virus positive	RSV	PIV (percentage of positive viral identifications)	Influenza	Adeno
Colombia 184	1985-86	IF	60	20	33%	75%	15%	5%	5%
Argentina 262	1984-87	culture & IF	1002	304	30%	60%	7%	5%	9%
Brazil 238	1987-89	culture & IF	657	272	41%	96%	0%	3%	0%
Uruguay 118	1987-88	culture & IF	204	74	36%	82%	7%	1%	4%
India 50	1985-87	culture & IF	328	86	26%	76%	15%	1%	5%
India 124	1985-87	culture & IF	809	342	42%	48%	27%	9%	9%
India 163	1987	IF	230	51	22%	27%	31%	18%	24%
Philippines 248	1984-86	culture, IF & serology	537	163	30%	40%	17%	9%	13%
Philippines 42	1990-92	culture & IF	322	85	26%	69%	4%	5%	22%
Hong Kong 237	1985-88	culture & IF	681	272	40%	86%			
Thailand 205	1986-87	culture, IF & serology	738	330	45%	43%	25%	14%	14%
Thailand 236	1988-89	IF	226	43	19%	93%	2%	2%	2%
Bangladesh 120	1986-88	culture & IF	601	127	21%	81%	4%	15%	4%
Bangladesh 206	1986-88	culture & IF	401	92	23%	63%	5%	16%	0%
Pakistan 82	1986-88	culture & IF	1492	553	37%	89%	2%	4%	5%
Gambia 77	1986-88	culture, IF & serology	74	25	34%	36%	8%	12%	28%
Gambia 76	1987-88	culture, IF & serology	90	42	47%	76%	7%	2%	10%
Gambia, well nourished 1	1990-92	culture & IF	119	48	40%	31%	13%	19%	48%
Gambia, malnourished 1	1990-92	culture & IF	158	55	35%	16%	18%	18%	49%
Madagascar 207	1983	IF	80	43	54%	23%	37%	30%	19%
Kenya 109,253	1981-82	culture & IF	835	451	54%	22%	4%	2%	4%
Philippines 217	1984	culture, IF & serology	308	198	64%	11%	14%	34%	6%
Singapore 67	1976-84	culture, IF & serology	NA	2232		33%	13%	43%	6%

Table 3 ct.

Country & reference	study year	method	number studied	number virus positive	% virus positive	RSV	PIV (percentage of positive viral identifications)	Influenza	Adeno
Papua New Guinea 199	1983-85	culture & IF	886	444	50%	31%	15%	5%	24%
Jamaica 123	1964-66	culture & serology	519	74	14%	12%	20%	23%	22%
Colombia 70	1972-73	culture & serology	155	93	60%	6%	16%	24%	12%
Colombia 30	1977	culture & serology	1229	250	20%	42%	11%	7%	34%
Panama 211	1983	culture & serology	340	29	9%	72%			21%
Papua New Guinea 222	1978-81	culture & serology	62	15	24%	27%	7%	13%	33%
Singapore 66	1977-79	culture & serology	1399	386	28%	30%	16%	32%	15%
Malaysia 193	1979-82	culture	180	52	29%	50%	12%	19%	4%
China 274	1981-83	culture & serology	369	148	40%	32%	8%	7%	30%
India 232	1981-82	culture	184	32	17%	6%	44%	16%	0%
Thailand 241	1986-87	culture	596	268	45%	45%	29%	9%	15%
South Africa 129	1966-72	culture & serology	3139	836	27%	23%	28%	14%	18%
Uganda 229	1972-73	serology & culture	662	180	27%	38%	32%	3%	15%

2.3.2 Epidemiology

2.3.2.1 Age distribution

Few community based studies have reported the age distribution of children with RSV infection.^{32,116,166,196,238,239} Only one study used immunofluorescence and documented only 11 cases with RSV. The study with the largest number of RSV cases followed a cohort of children in Colombia from birth to 17 months of age.³² Forty percent of RSV cases were found in the first 6 months of life, and 46% in the second half year. Overall, there is insufficient information on the age distribution of children affected by RSV in the community.

In hospital based in- or outpatient studies, the proportion of children aged between birth and 5 months of age with RSV infection varied between 9%¹⁹² and 87%.⁵⁷ Taking only studies which included immunofluorescence or antigen detection, and which reported on children up to at least 5 years of age,^{2,49,67,82,109,120,238,248} on average 39% (range 20-62%) were under 6 months of age. On average, children aged between 6 and 11 months of age comprised 24% of cases (range from 14-38%). Thus, on average, 63% of children were under 1 year of age. Three additional studies which did not differentiate in the age group under 1 years of age found 62% of children under one year of age.^{115,124,184} On average, 20% (range 13-29%) of the children were between 1 and 2 years of age.

2.3.2.2 Incidence rates of RSV-ALRI

Few studies attempted to quantify the incidence rates of RSV-ALRI.^{30,32,57,108,238} The reported incidence rates vary between 10/1000 children under 1 year of life for hospitalisation with RSV-ALRI in southern Israel⁵⁷, to 198/1000 child years for the age group between birth and 18 months in a community based study from Colombia.³²

2.3.2.3 Sex distribution

Several studies have reported on the sex distribution of the patients.

^{2,54,69,76,115,121,122,131,185,196,207,236,238,251} Twelve out of 14 studies found a male predominance; on average 60% of infected children were male (range 43-88%).

This male preponderance corresponds to the generally higher incidence of ARI of any aetiology in boys.²¹¹

2.3.2.4 Seasonality

In most published studies, RSV has been a highly seasonal infection. RSV outbreaks in areas with temperate or Mediterranean climates in the southern hemisphere appear mainly during the cold months, as is the case in Western Europe and North America,^{83,153}. This temperature dependent pattern appears to be independent of the rainfall pattern, as winter months have high rainfall in places such as Santiago, Chile,¹⁸ but low rainfall in places such as Johannesburg in South Africa¹²⁹ (Figures 3 and 4). In desert climates such as Kuwait¹¹⁵ or Saudi Arabia,¹²² cases are seen also in the cold months.

In areas with tropical or sub tropical climates and seasonal rainfall, RSV outbreaks are associated frequently with the rainy season, and not with the colder season (Figures 5-9). The peak of RSV transmission is usually 1 to 2 months after the onset of the rains.

In countries close to the equator with perennial high rainfall, such as Singapore,^{66,251} Colombia,³⁰ or islands such as Hawaii,²¹⁰ the situation is less clear cut.

These countries have a distinct pattern of RSV transmission, with most cases appearing in one half of the year, but not in the other. But neither temperature nor rainfall appear to be the main determinant of the timing of these outbreaks.

Studies from the north of the Indian subcontinent have shown contradictory patterns: Islamabad in Pakistan and Chandigarh in India have similar climates, and are only 500 km apart, but they show different seasonality patterns.

Outbreaks in Pakistan were reported to be mainly in the cold season,⁸² whereas

an outbreak described in Chandigarh was associated with the rainy season.³ The situation is similar with regard to Calcutta in West Bengal and Dhaka in Bangladesh: outbreaks in Calcutta were reported mainly in the rainy season,^{116,138,196} whereas most of the cases from Bangladesh appeared in the drier, colder season.¹²⁰

Figures of seasonality of RSV disease

Each graph depicts the mean monthly rainfall, the mean monthly maximum and the mean monthly minimum temperature as lines, and series of cases as bars with different hatches. The meteorological data were retrieved from the US National Climatic Data Center. ¹⁸¹

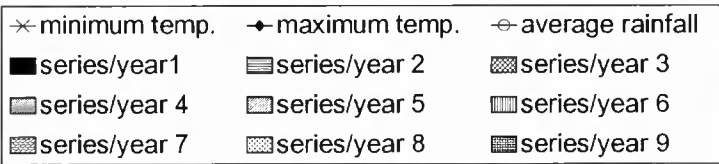
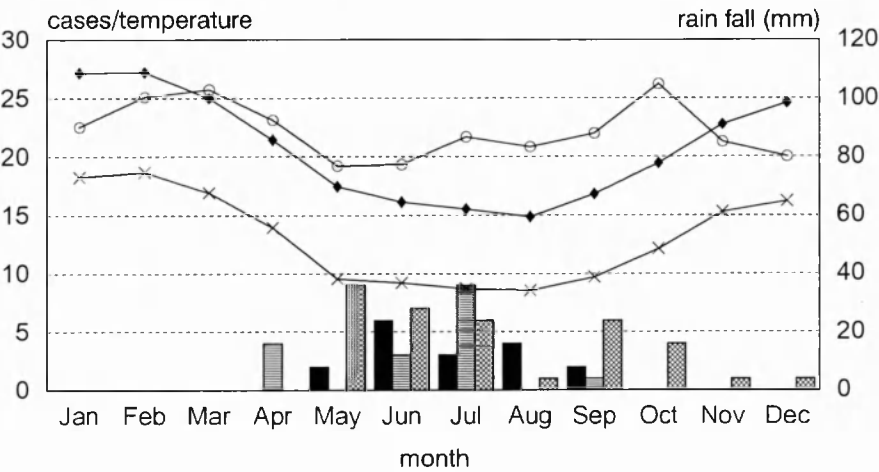


Figure 2: Uruguay 1985-1987;²¹⁵ Argentina 1984-1987.²⁶²

Uruguay, Montevideo, 35°S



Argentina, Buenos Aires, 35°S

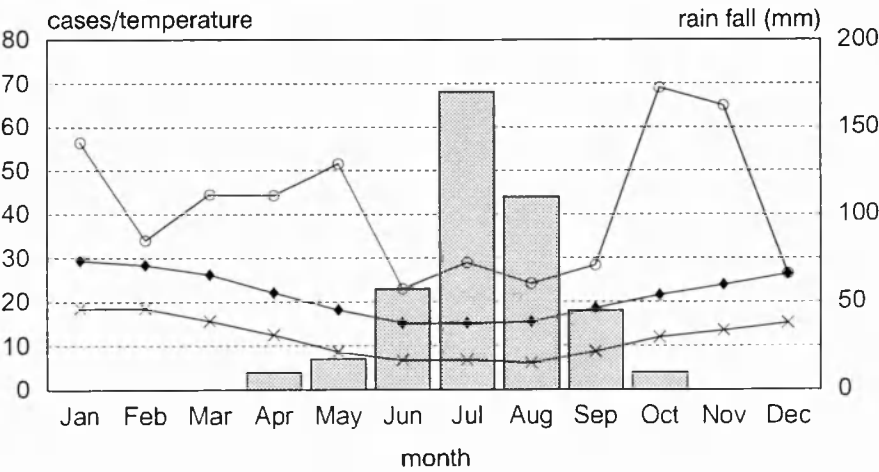
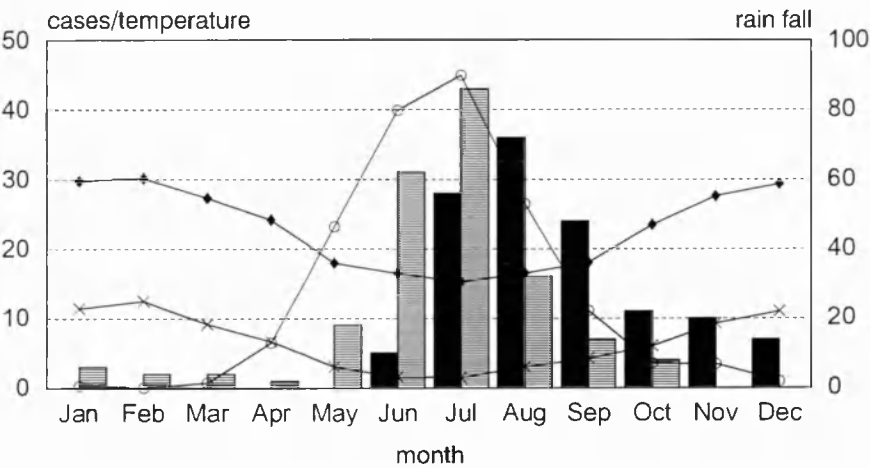
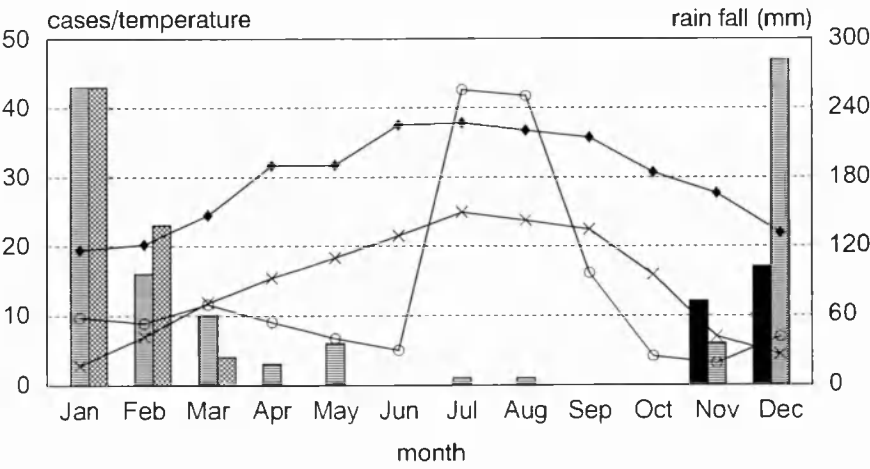


Figure 3: Chile 1988-1989;¹⁸ Pakistan 1986-1988;⁸² India 1968.³

Chile, Santiago, 35°S



Pakistan, Islamabad, 33°N



India, Chandigarh, 31°N

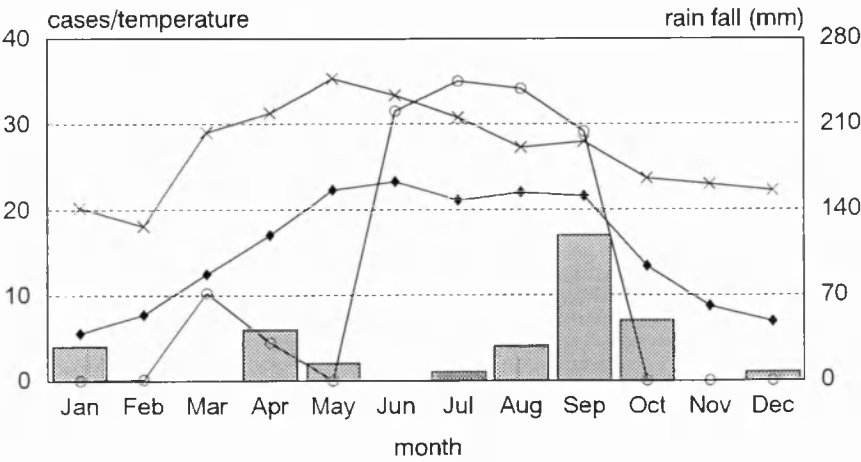
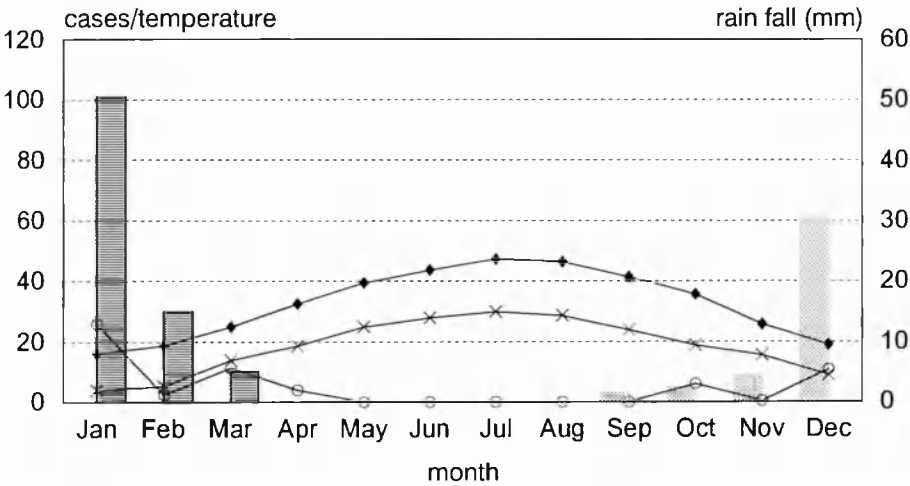
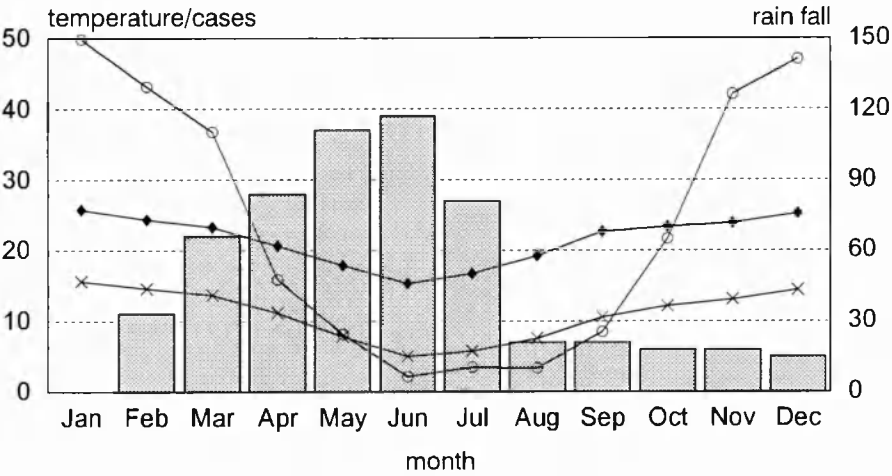


Figure 4: Kuwait 1993-1994;¹¹⁵ South Africa 1966-1972;¹²⁹ Saudi Arabia 1991-1992.¹²²

Kuwait, 30°N



South Africa, Johannesburg, 26°S



Saudi Arabia, Riyadh, 25°N

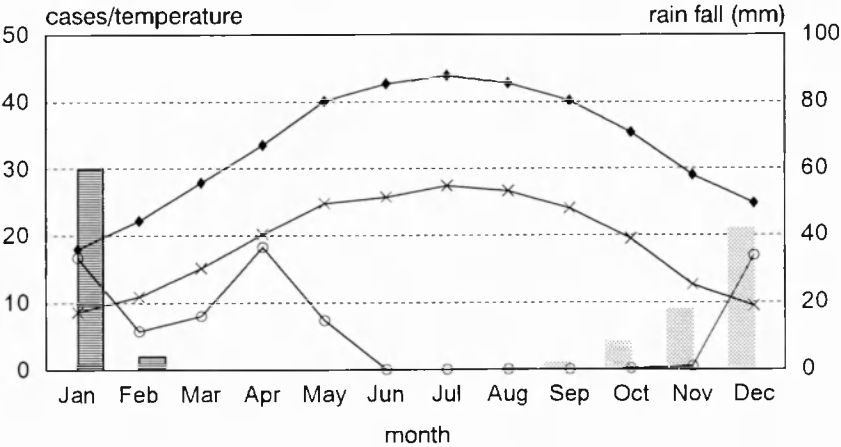
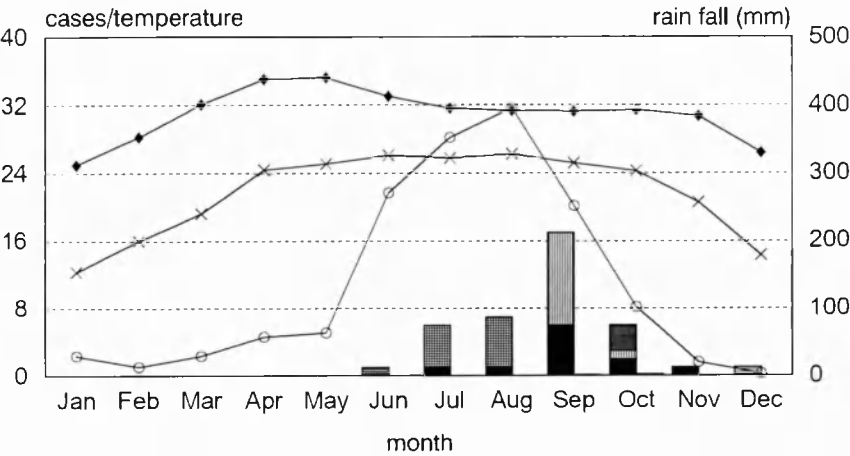
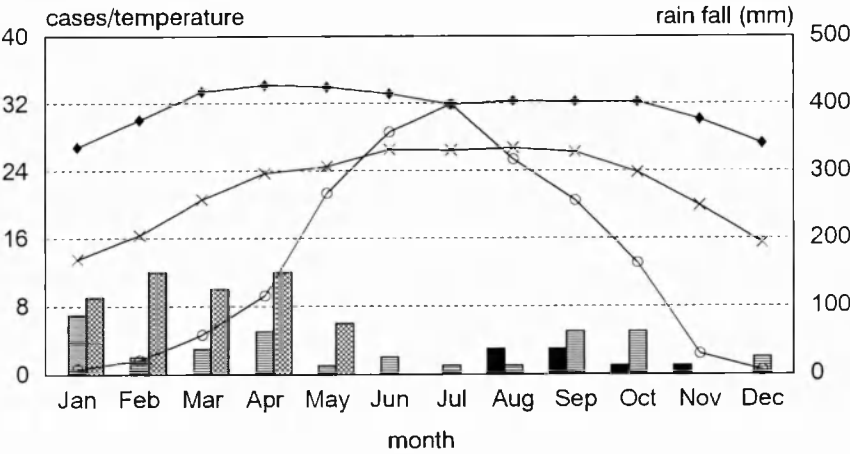


Figure 5: India 1964-1966;¹³⁸ Bangladesh 1986-1988;¹²⁰ China 1978-1979.²²³

India, Calcutta, 23°N



Bangladesh, Dhaka, 23°N



China, Guangzhou, 22°N

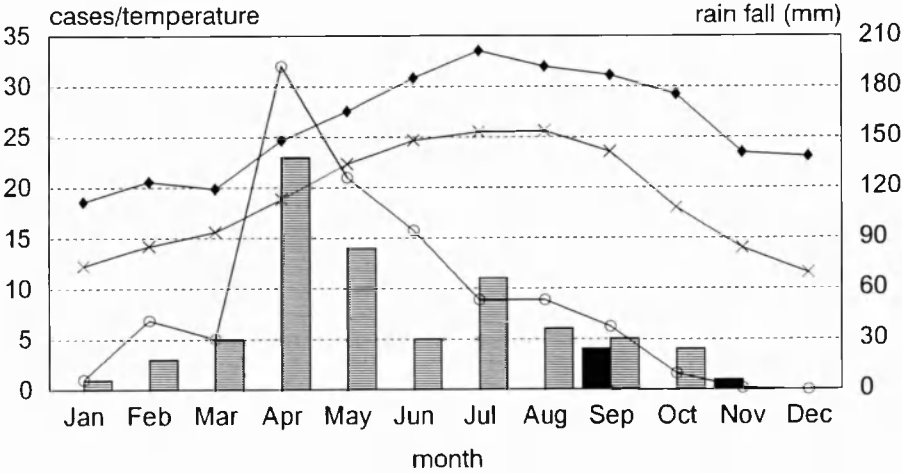
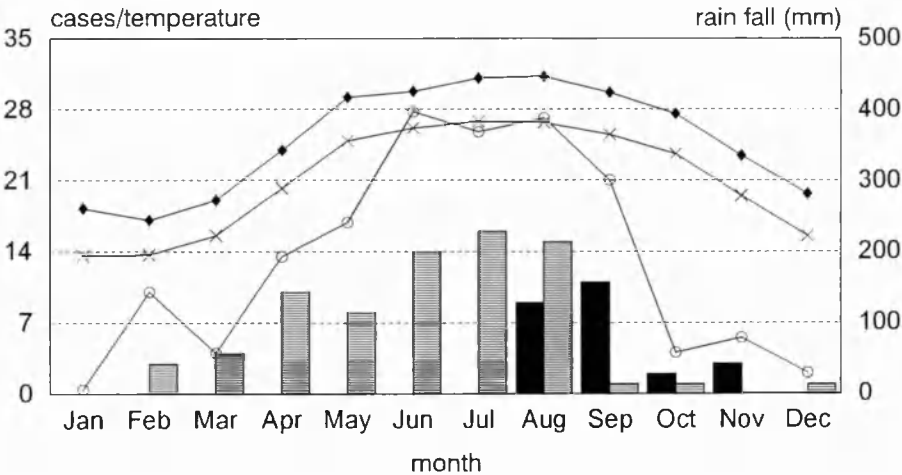
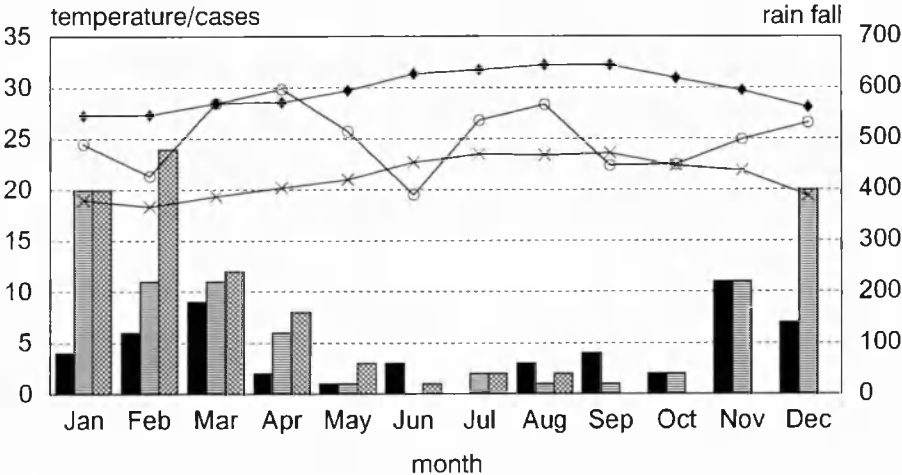


Figure 6: Hong Kong 1985-1986;²³⁷ Hawaii 1987-1989;²¹⁰ Philippines 1984²¹⁷, 1985-1986.²⁴⁸

Hong Kong, 22°N



Hawaii, 20°N



Philippines, Manila, 14°N

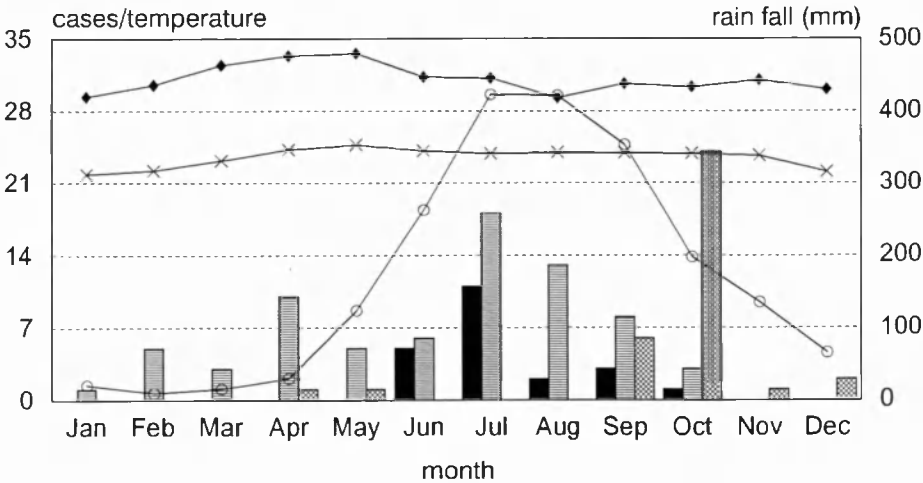
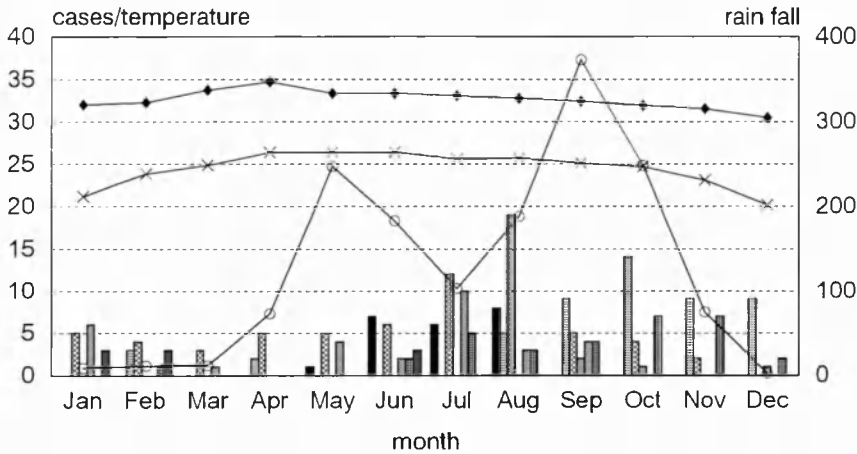
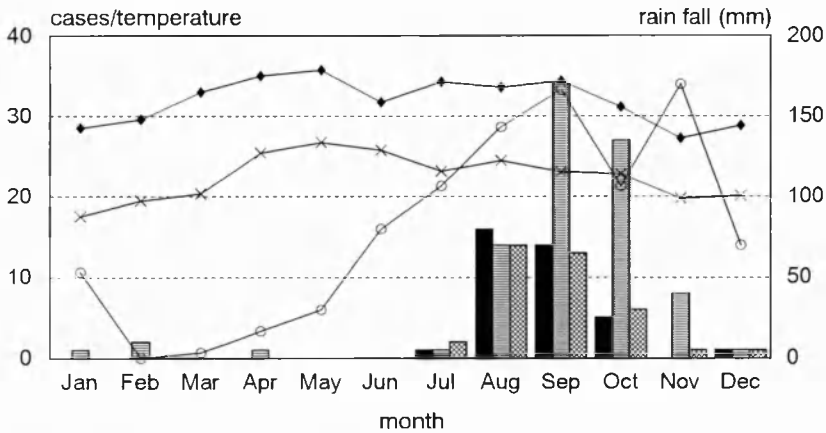


Figure 7: Thailand 1968,¹⁹¹ 1986-1987,²⁴¹ 1986-1987,²⁵⁰ 1988-1989;²³⁶ India 1985-1987;¹²⁴ Trinidad 1964-1966.²³⁰

Thailand, Bangkok, 13°N



India, Vellore, 12°N



Trinidad, Port of Spain, 10°N

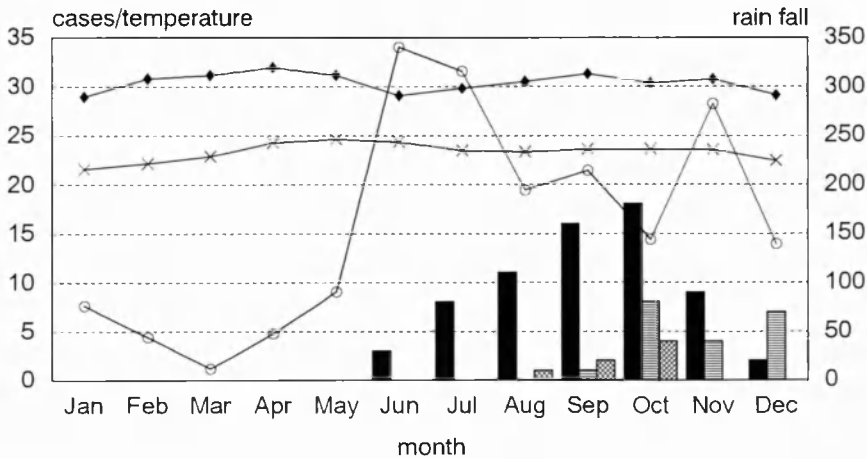
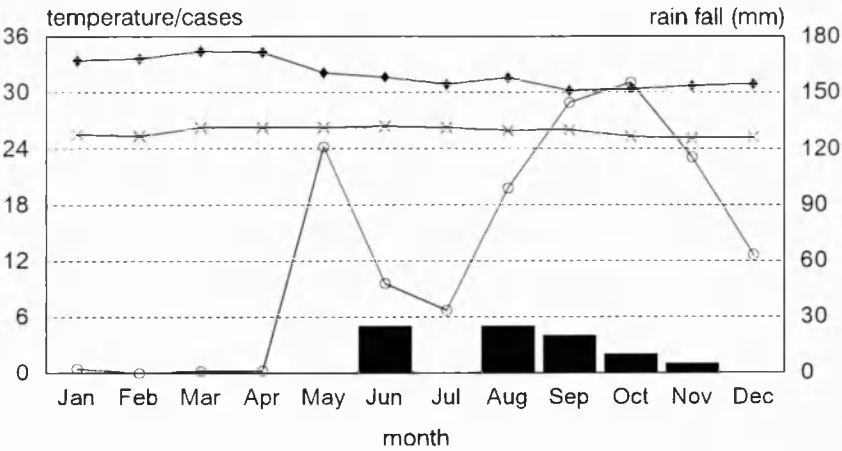
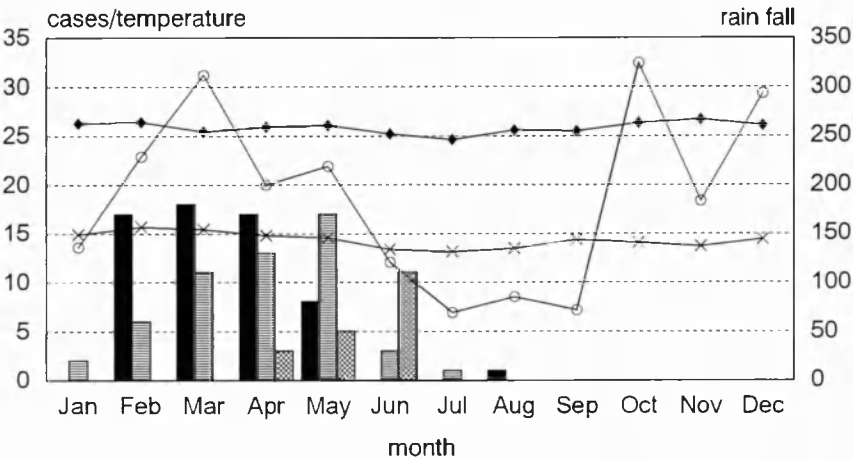


Figure 8. Panama 1983;²¹¹ Papua New Guinea 1983-85;¹⁹⁹ Colombia 1977-1979.³⁰

Panama, 9°N



Papua New Guinea, Goroka, 6 °S



Colombia, Cali, 4°N

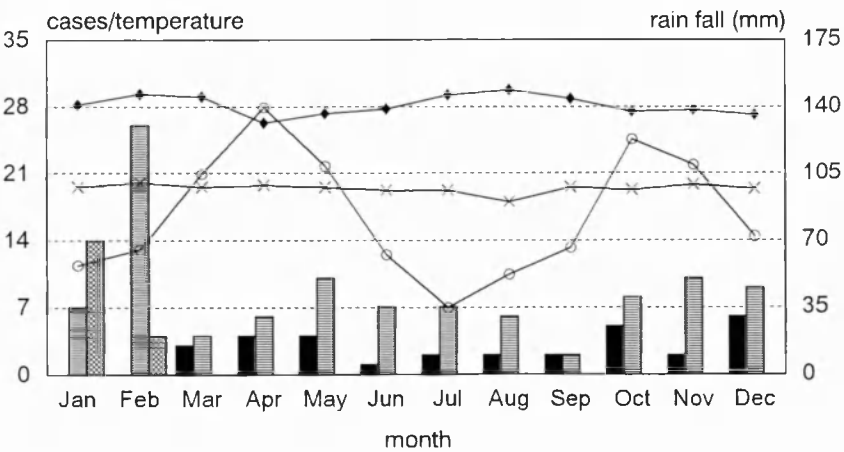
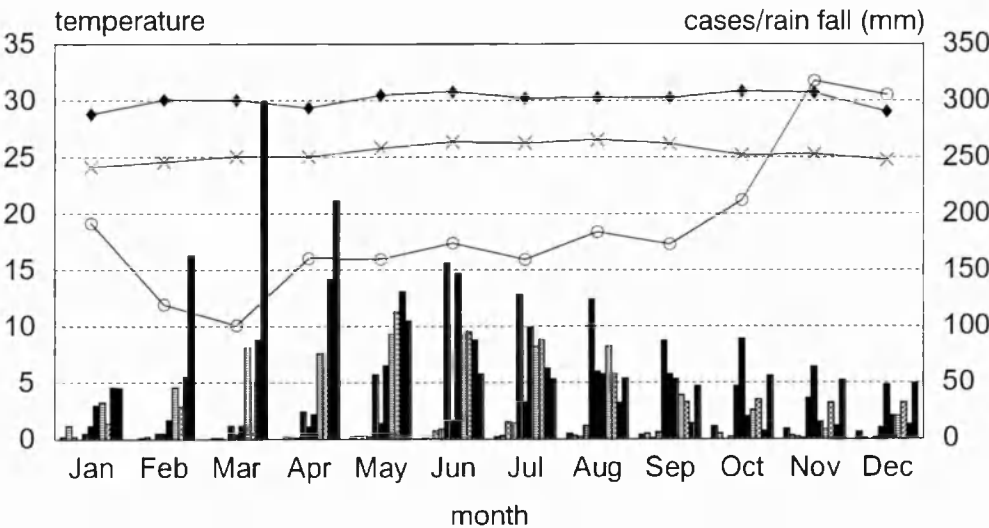
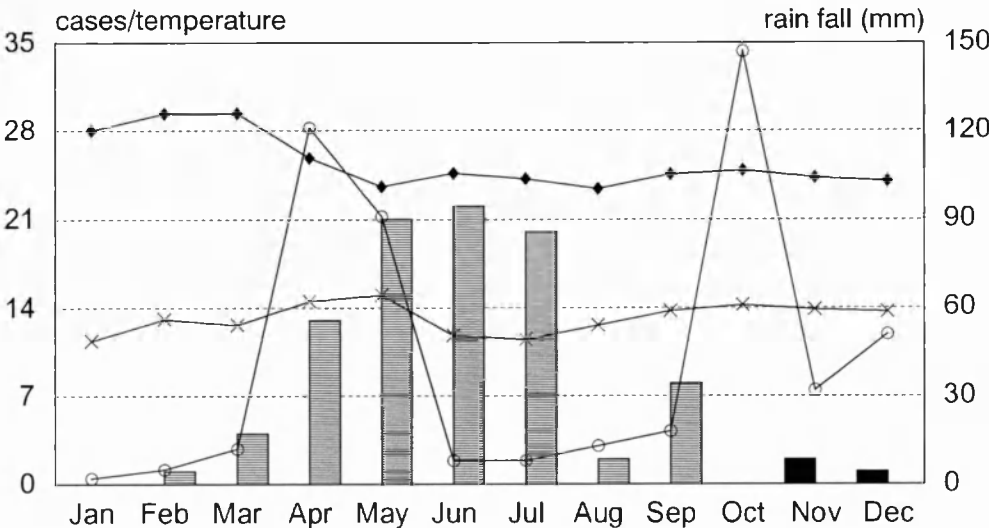


Figure 9: Singapore 1977-1979,⁶⁶ 1980,²⁵¹ 1989-1995; Kenya 1981-1982.
109,253

Singapore, 4°N



Kenya, Nairobi, 2°S



2.3.3 Clinical features

2.3.3.1 Bacterial co-infections

Twelve studies looked for concurrent bacterial infections in cases of RSV infection.^{1,2,50,76,82,115,118,124,206,222,248,253} The isolation rate of bacteria varied

between 2%^{50,124} and 50%.²²² Most studies were very small, involving less than one hundred patients with RSV infection. A study from the Gambia, where the bacterial co-infection rate was 22%,⁷⁶ included serology as well as culture as an indicator of bacterial infection. The only large study with a high bacterial isolation rate was that conducted in Pakistan⁸², in which bacteria were found in 31% of all cases of RSV infection. In most studies, *S. pneumoniae* was the most frequently isolated organism, followed by *H. influenzae*.

2.3.3.2 Malnutrition

Two studies from Africa report the influence of malnutrition on the prevalence of RSV. In a study from the Gambia,¹ where malnourished and well-nourished children with pneumonia were investigated concurrently, RSV was found in 13% of well nourished children with ARI, but in only 6% of malnourished children investigated. In a study from Nigeria,¹⁸⁶ RSV was found in 16% of malnourished children with ARI, but in 55% of well nourished controls. In Papua New Guinea, Philipps et al¹⁹⁹ did not find any association between RSV infection and malnutrition. These observations suggest that malnutrition is less of a risk factor for the development of severe RSV infection than for respiratory infections of other aetiologies.

2.3.3.3 Mortality

Fifteen studies^{2,18,30,32,43,50,57,69,115,118,122,185,217,237,251} report case fatality for RSV infection; this ranged from 0% to 6%. The majority of the hospital-based studies (8 of 14) did not report any deaths. A study from Argentina,⁴³ which investigated 31 deaths from ARI, found RSV in 4 children; 2 of whom were

preterm, and one of whom had a combination of RSV, adenovirus and *Streptococcus viridans* infection. In 4 out of 11 cases in whom no pathogen could be identified, a clinical diagnosis of bronchiolitis was made. On analysis of all studies reporting deaths, an association with a bacterial pathogen was made in 3 of the 18 reported RSV deaths (16%): two with *Staphylococcus aureus*,^{30,50} and the already mentioned streptococcal infection. Six children (33%) had additional risk factors: 2 were preterm babies,⁴³ one had a low birth weight,⁵⁰ one had a ventricular septal defect, and two had neurological disease.¹¹⁵

Two studies from the south Pacific, which report cases of bronchiolitis without virological diagnosis, reported a case fatality rate of 2%³⁴ and 11%,¹⁰⁸ respectively. A study from India¹³³ found a case fatality rate of 8.3% for clinical bronchiolitis, without determining the aetiology. Studies from Bangladesh^{120,206} and the Philippines²⁴⁸ report the case fatality rate of viral ARI to be much lower than bacterial ARI, 3% versus 7%, without specifying RSV explicitly.

The impact of RSV on mortality in the community cannot be assessed from the published literature.

2.3.3.4 Further wheezing

In developed countries, there is debate about whether RSV triggers further episodes of wheezing. There is only one published study from a developing country, Qatar,¹⁹⁵ which examines this issue. It found that 44% of children with RSV bronchiolitis had further episodes of wheezing over the next two years, in comparison to only 13% of controls.

2.3.4 Management

WHO has published guidelines for the treatment of acute lower respiratory tract infection in small hospitals and outpatient facilities.²⁶⁷ As an aetiological diagnosis is normally not possible in these settings, these are meant mainly to reduce mortality from bacterial infection through appropriate use of antibiotics. Further guidelines specify indications and practicalities of oxygen delivery which may be particularly important in children with RSV infection.²⁶⁸

Table 4: Aetiology of ALRI in different studies from The Gambia

Author and study years	group studied	number studied	bacteria positive	<i>S. pneumoniae</i>	<i>H. influenzae</i>	other	virus positive	RSV	Influenza A & B	PIV	Adeno
Wall 254 1982-84	< 10 years, lobar consolidation, hospital	51	35 (69%)	26 (51%)	12 (24%)	<i>S. aureus</i> 1 <i>B. catarrhalis</i> 2			not done		
Forge 76 1987-88	< 1 year, hospital	90	27 (30%)*	18 (20%)	10 (11%)	<i>M. catarrhalis</i> 2 <i>Salmonella. sp.</i> 1	42 (47%)	32 (37%)	1 (1%)	3 (3%)	4 (4%)
Forge 77 1986-88	1-9 years, hospital	74	57 (77%)*	39 (61%)	10 (16%)	<i>M. catarrhalis</i> 4 <i>S. aureus</i> 4	25 (37%)	9 (12%)	3 (4%)	2 (3%)	7 (9%)
Forge 75 1987-88	community, < 5 years	222 episodes	32 (14%)	19 (9%)	10 (5%)	<i>M. catarrhalis</i> 6	55 (25%)	4 (2%)	14 (6%)	14 (6%)	18 (8%)
Adegbola 1 1990-92	3 months - 5 years malnourished	159	28 (18%)	11 (7%)	6 (4%)	<i>Salmonella sp.</i> 4 <i>M. tuberculosis</i> 4	55 (35%)	9 (6%)	10 (6%)	10 (6%)	27 (17%)
	3 months - 5 years well nourished	119	42 (35%)	31 (26%)	8 (7%)		48 (40%)	15 (13%)	9 (8%)	6 (5%)	23 (19%)
Mulholland 174 1990-92	< 3 months "sepsis"	413	33 (8%)**	3 (0.7%)	0	<i>S. aureus</i> 17 <i>Salmonella sp.</i> 5 <i>E. coli</i> 3	128 (29%)	51 (12%)	68 (16%)	26 (6%)	16 (4%)
O'Dempsey 187 1989-91	pneumonia < 5 years	1014	117 (12%)**	81 (8%)	18 (2%)	<i>Salmonella sp.</i> 12 <i>S. aureus</i> 3			not done		
Usen 249 1993-95	pneumonia 2 months - 3 years	1821	201 (11%)**	99 (3%)	29 (2%)	<i>S. aureus</i> 22 <i>Salmonella sp.</i> 21 <i>E. coli</i> 9 other 21			not done		

Note: Percentages are % of all children studied

* Includes serology and antigen detection for the identification of bacteria

** excluding meningitis

No clear pattern of seasonality was discernible. One large outbreak of RSV occurred in the early dry season, the other in the wet season. Outbreaks were separated by 2 ½ years of irregular cases of bronchiolitis, but no virology was done during this period. RSV was not isolated, and serological evidence for infection found in only 4 cases during one entire year in a community study in Basse in Upper River Division. These confusing epidemiological data established the need for a more detailed and comprehensive study.

Chapter 3. Materials and Methods

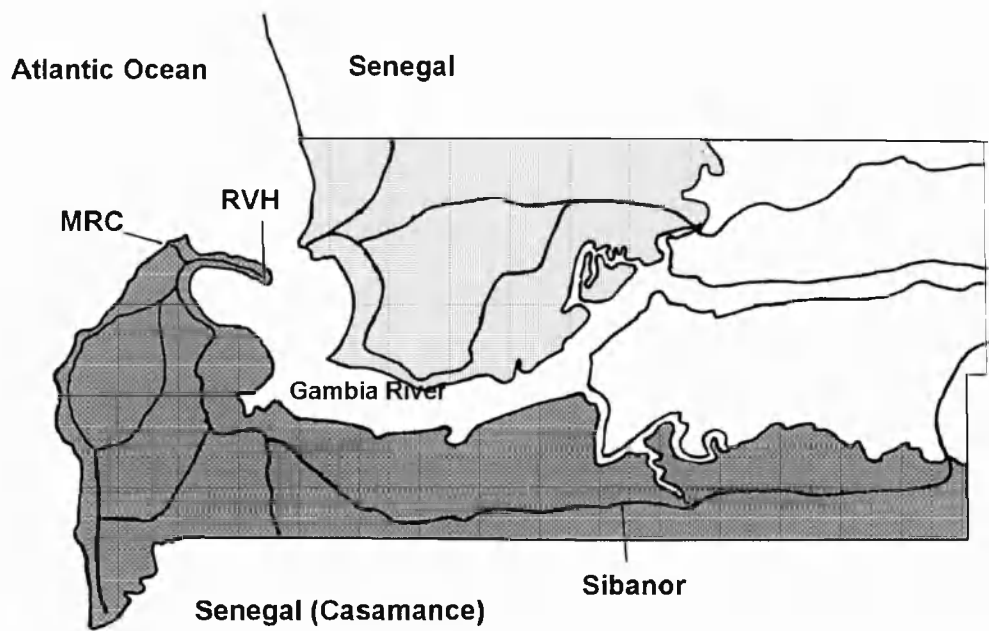
3.1 *The Gambia*

3.1.1 Geography

The Gambia is a small country on the west coast of Africa approximately 13° north of the equator. The country follows the river from which it derives its name for a distance of about 350 km inland from the sea. The maximal width is at the coast where it is 48 km across, but it narrows further inland to about 21 km. The total area of The Gambia is 10367 km². Except for the coast line, the country is totally surrounded by Senegal. The land is generally flat, consisting of a sandstone plateau through which the river has carved its course. The river is bounded by plains which are saline in the lower reaches and therefore covered by mangroves and unsuitable for farming. Further upriver, the river plains are flooded in the rainy season and used for rice farming.

Administratively, The Gambia is divided into 5 Divisions and 2 Municipal Areas. The two municipal areas are Banjul and Kanifing, each including the periurban areas around the coast. The divisions (seat of divisional administration) are Western Division (Brikama), Lower River Division (Mansa Konko), Central River Division (Janjangbureh), Upper River Division (Basse), and North Bank Division (Kerewan). The two municipal areas, Western Division and the western part of North Bank Division form the Western Region, the geographic area of this study.

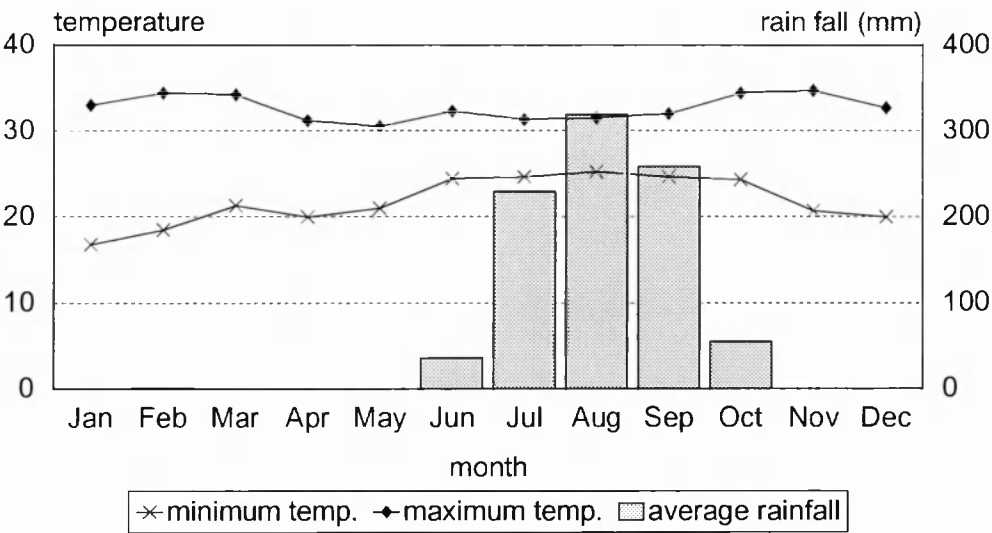
Figure 11: Map of The Gambia indicating the study area and the three main study hospitals, the Royal Victoria Hospital in Banjul, the MRC hospital in Fajara, and the WEC Mission hospital in Sibanor.



3.1.2 Climate

The climate of the Gambia is tropical with two distinct seasons: a dry season from November to June, and a wet season from June to October. The mean monthly figures for rain fall and temperature, as measured in Yundum at the coast, are shown in Figure 12.

Figure 12: Mean monthly rain fall and maximum and minimum temperatures as measured at Yundum airport, Western Gambia.

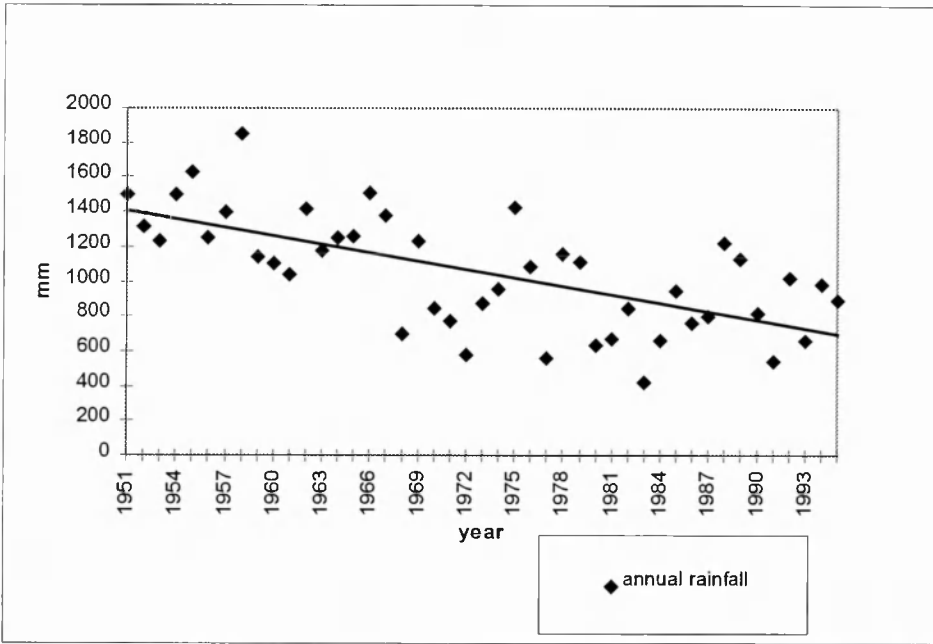


Note: The values are averaged for the years from 1990 to 1995.

The maximum daily temperature at the coast is relatively stable around the year. The average minimum temperature rises from minimal values in December of 16° to 25° in August. Further away from the coast, maximum temperatures rise above 40° in the middle of the dry season.

The climate has become progressively drier over the last 50 years, the average annual rainfall has decreased from around 1400 mm per year in 1950 to around 700 mm per year more recently. This trend is shown in Figure 13.

Figure 13: Annual rainfall as measured at Yundum between 1951 an 1995



3.1.3 Population

The population of the Gambia rose above 1 million for the first time in the 1993 census.¹³ The highest concentration of the population is in the periurban areas of Banjul, Serekunda, and Brikama. About half of the population live in the Western Region. The annual growth rate of the population as calculated from the 1993 census is 4.2 %, part of which is a result of immigration rather than through births.

The main ethic groups in The Gambia are Mandinka, Wolof, Jola, Fula and Serahule. The vast majority of Gambians are Muslim.

3.1.3.1 Childhood population in the Western Region

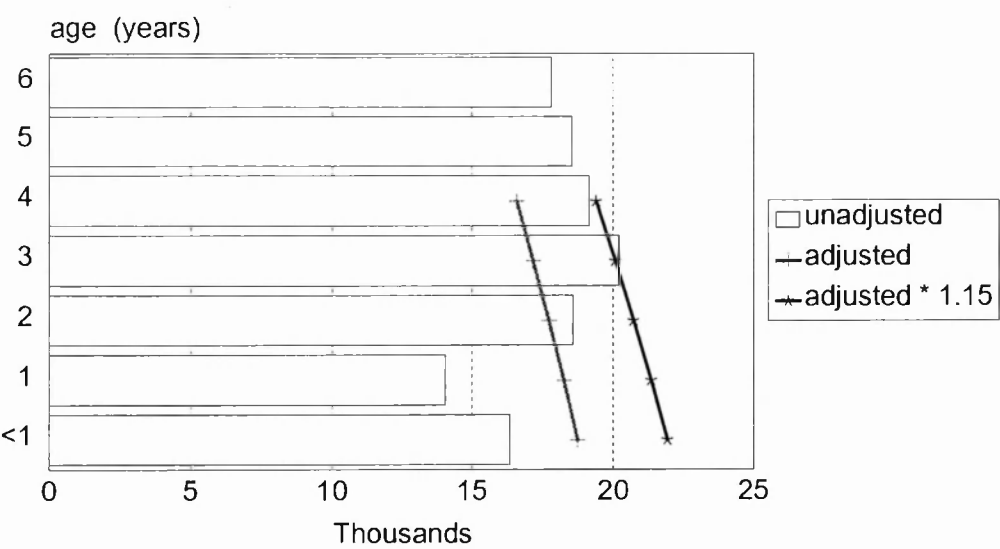
The following table gives details of the population under 7 years of age provided by the 1993 census.

Table 5: Population in early childhood by district in the Western Region of the Gambia, as reported in the 1993 census.

age group (years)	< 1	1	2	3	4	5	6
Banjul	981	779	1008	1021	1025	956	882
Kanifing	6094	5287	6695	7205	6700	6302	6061
Kombo North	2637	2156	2810	3040	2874	2822	2656
Kombo South	1256	1080	1503	1639	1469	1547	1381
Kombo Central	1641	1487	1871	2047	1961	1844	1826
Kombo East	604	578	770	798	792	770	803
Foni Brefet	248	205	295	316	293	359	316
Foni Bintang	317	275	402	455	499	459	472
Foni Kansala	170	199	245	323	307	318	309
Foni Bondali	110	106	148	171	181	172	200
Foni Jarrol	129	125	159	196	203	217	189
Western Division	7112	6211	8203	8985	8579	8508	8152
Lower Niumi	1047	857	1269	1422	1329	1368	1239
Upper Niumi	688	545	855	893	884	840	856
Jokadu	452	397	594	710	679	601	657
North Bank West	2187	1799	2718	3025	2892	2809	2752
Total Western Region	16374	14076	18624	20236	19196	18575	17847

In this data set, the number of children in the lower age categories is lower than in higher age categories, which does not accord with expectation. Younger children must have been under-reported, a common feature in censuses in developing countries (A. Hill, personal communication). Computer programmes have been developed to adjust mis-reporting between age categories and to create a smooth pyramidal age distribution keeping the total number of persons reported in the census constant. Figure 14 shows the raw data for children in the Western Region, and after adjustment by the Arriaga method.^{17,224} As this method does not adjust for underreporting, a second line is depicted which uses the adjustment and adds another 15% for underreporting to the number of children.

Figure 14: Number of children in yearly age groups in the Western Region of the Gambia. Depicted are the raw data, the data adjusted by the Arriaga method,¹⁷ and after a further adjustment of 15%.



3.1.4 Health system

The Ministry of Health, Social Welfare, and Women’s Affairs is responsible for health care in the country. For each division, health care is co-ordinated by a Divisional Health Team. The lowest level of health care is provided by Village Health Workers and Traditional Birth Attendants. These are generally illiterate, but have been trained in courses of 6-8 weeks duration to treat minor illness and refer more severe cases to a health centre. They are supervised by community health nurses. Minor health centres are based at bigger villages or small towns. These are manned by nurses, nurse midwives and public health inspectors. They are the first point of referral, provide preventive services such as vaccination and antenatal care, curative services and deliveries. They generally have a small number of beds for inpatient care, but are not encouraged to retain patients with

more severe conditions such as severe pneumonia, meningitis or cerebral malaria. Major health centres generally have a doctor in charge, a larger number of beds for inpatient care and are able to deal with the more severe conditions mentioned above. In the Western Region, they do not have facilities for major operations or operative deliveries. The next level of referral are two hospitals, one in Bansang in Central River Division, and the Royal Victoria Hospital in Banjul, the only paediatric referral hospital in the country.

Outside the Government sector, health facilities are run by private practitioners mainly in the periurban areas, and by non-governmental organisations. The MRC maintains a outpatient clinic and 40 bedded hospital caring for medical and paediatric conditions. In Sibanor, a Protestant mission society (WEC) runs a major health centre with 38 inpatient beds.

3.2 Study methods

3.2.1 Clinical spectrum and epidemiology

3.2.1.1 Patients

Surveillance for RSV disease was undertaken in three hospitals in the Western Region of the Gambia: the Royal Victoria Hospital (RVH) in Banjul on the coast, the MRC Hospital in Fajara, approximately 15 km from Banjul, and Sibanor Mission Hospital 80 km inland. The RVH is the paediatric referral hospital for the entire western half of The Gambia. The MRC hospital serves a mainly periurban population, whereas Sibanor serves a mainly rural population. Surveillance started at RVH and MRC in October 1993, in Sibanor in January 1994, and ended for the purpose of this thesis in December 1996. Children admitted to these

hospitals were screened for RSV disease if they were less than 2 years of age and had any form of acute lower respiratory infection, if they received oxygen for any condition, or if one of the attending physicians specifically requested that the child should be screened. A considerable proportion of the children investigated were enrolled in a *H. influenzae* type b vaccine trial started in April 1993.¹⁷⁶ Outpatients were only enrolled if a study physician was present.

3.2.1.2 Sample collection

Nasopharyngeal aspirates were obtained from children in the study on each working day by a trained field worker. Samples from children admitted on a holiday were obtained on the next working day. A size 8 French suction catheter (Sherwood Medical, Tullamore, Ireland) was introduced through one nostril into the nasopharynx. The catheter was connected to a mucus trap (Mucus Specimen Set, Bibby Sterilin Ltd., Stone, Staffs, UK) and suction was applied with a manual suction pump (Manuvac, Weinmann, Hamburg, Germany). After a sufficient sample of mucus had been obtained, the catheter was washed through with 2 ml of sterile phosphate buffered saline and the specimen was transported to the laboratory on a cold pack in a cold box at 4 °C.

Children enrolled in the Hib conjugate vaccine trial and children admitted to MRC had blood cultures taken on admission. Blood cultures were not done routinely in Sibanor and RVH. Samples were collected by venepuncture into a syringe and inoculated into brain heart infusion and tryptic soy broth immediately. Until transport to the main laboratory, blood culture bottles were stored in an incubator at 37°C. Further processing of blood cultures and isolation of organisms followed routine procedures as described previously.¹

3.2.1.3 Data collection

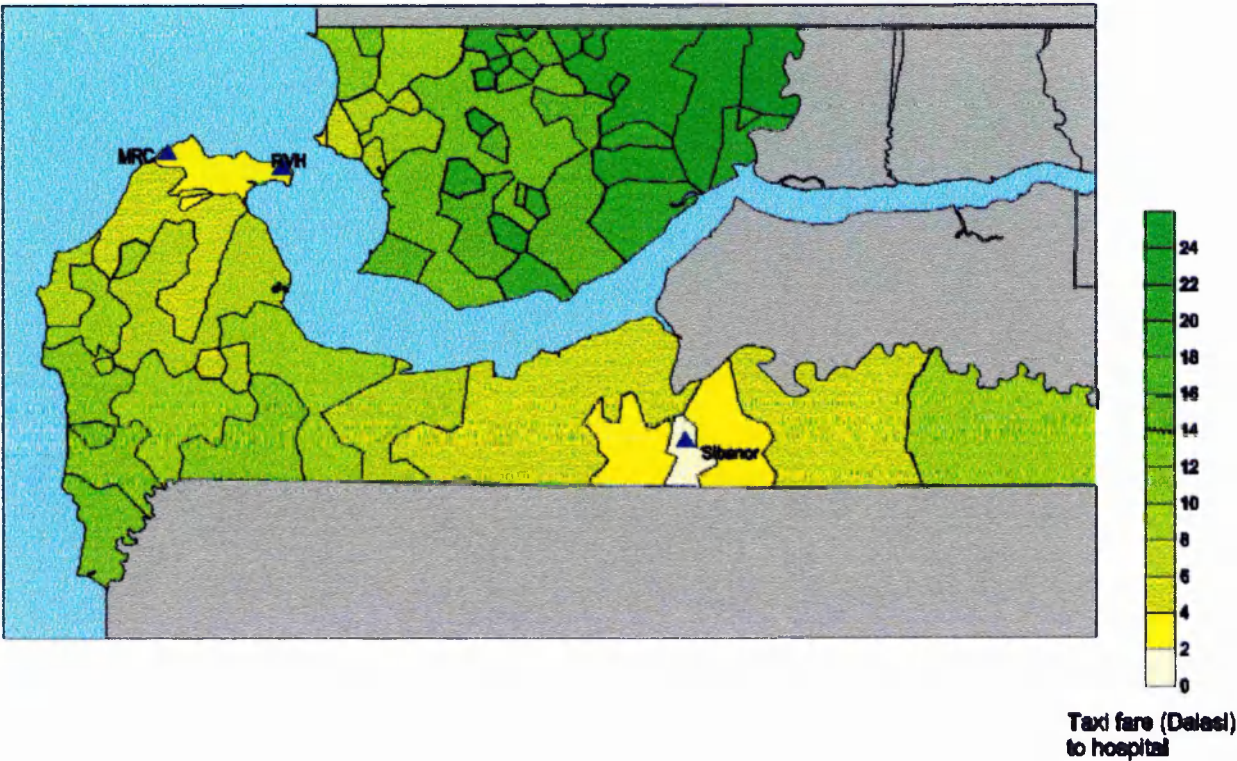
After patients were identified as RSV positive, data were collected from the medical records. Children enrolled into the Hib vaccine trial and other formal studies in progress at that time ²⁵⁹⁻²⁶¹ had history and physical examination documented in a standardised fashion and these data were used for the documentation of signs and symptoms (n=247). In other cases, clinical details were extracted retrospectively from admission or outpatients records. A data base was constructed covering the history and a range of vital and respiratory signs such as crepitations (crackles) and wheeze for each patient. ⁵⁵ If a complete physical examination was documented in the case notes, signs not mentioned specifically were considered absent. Outcome and duration of stay in hospital were noted from the patients records.

3.2.1.4 Calculation of incidence rates

The number of hospital admissions in the three study hospitals was obtained from routine records in hospital discharge books. Census data were obtained from the Department of Statistics, Government of The Gambia, for towns and villages in the study area. The age pyramid was smoothed, ¹⁷ but underreporting of young infants was not adjusted further. In this way, the number of children in yearly age groups was obtained for each of the 498 villages and settlements in the Western Region. Incidence rates were calculated for admissions with a discharge diagnosis of ALRI, RSV-ALRI, and RSV infection with hypoxaemia. As the study covered only part of 1993 and involved only 2 study hospitals in that year, incidence rates were calculated only for 1994 to 1996. One way transport fares by public transport to the study hospitals were obtained

for each village, and the transport cost was used to stratify and adjust incidence rates by distance (Figure 15). For the unadjusted incidence rates, villages were grouped by transport fare into 5 groups: 0-2 Dalasis, 3-5 Dalasis, 6-10 Dalasis, 11- 15 Dalasis, and above 15 Dalasis (10 Dalasis = 1 US\$). The childhood population in each age category resident in villages falling into each transport cost group was summed up and used as the denominator for the summed number of cases originating from these villages. Details on the calculation of adjusted incidence rates by Poisson regression are given below in Chapter 3.2.6.2.

Figure 1: Contour map of the western region of The Gambia showing the minimum transport fare (one way) to one of the study hospitals.



3.2.2 Community studies

Children were identified in hospital as described above. As soon as an index case was confirmed, usually within a week of admission, a field worker visited the compound of the index child and obtained consent from the compound head for a visit by the field team. The compound is the usual unit of living in the Gambia, where mostly members of an extended family share a common central space and some social tasks such as cooking. The compound corresponds to an extended household. Children 5 years of age or below were examined for the presence of respiratory infections. If a respiratory infection (upper or lower) was diagnosed, a nasopharyngeal aspirate was obtained from the child and stored and transported to the lab on ice. A serum sample was obtained on the initial visit and on a final visit 6 weeks later. In between these two visits, the compound was visited by a field worker twice a week, and nasopharyngeal aspirate samples were obtained from any child who had developed a fresh respiratory tract infection. During the 1994 RSV-season, a neighbouring compound was investigated in the same manner as the compound of the index case.

3.2.3 Risk factors study

Cases were detected through surveillance for RSV disease as described above. Children were included as cases if they were admitted to hospital in 1993 or 1994, were under one year of age, had a mother or guardian who was willing to answer the risk factor questionnaire, and who lived within approximately 40 km from one of the three study hospitals in the Western Region of the Gambia. After a case had been identified, a field worker interviewed the mother about a range of potential social and environmental risk factors, using a standard questionnaire. During 1993, controls

were enrolled from a follow-up cohort of the Gambian Hib vaccine trial.¹⁷⁶ Eligible for selection as controls were children registered in the same health centre as the case, matched for date of birth within a week, but who were not admitted with an ALRI during the time of the RSV outbreak during which the case presented. From 1994, the questionnaire was extended to cover additional more immediate risk factors such as feeding, the type of meals, exposure to smoke and animals, and details of children on the compound. Controls for cases from 1994 onwards were enrolled after the outbreak from amongst children living in the same village or suburb, matched for age, who were not admitted to hospital during the outbreak with an acute respiratory tract infection. A field worker was visiting the nearest compound, inquired about children meeting above conditions and whether the parents were willing to participate. If either of this was not the case, he continued until both conditions could be met. Controls were enrolled and parents interviewed in the early dry season, whereas cases were mostly enrolled in the rainy season. Each case child was matched with at least one control child, but none with more than 2 control children. The risk factors covered in the questionnaire are shown in Table 6.

Table 6: Potential risk factors for severe RSV disease covered in the questionnaire.

<u>About the mother</u>		
Alive/dead	Age	Nationality
Ethnic group	Occupation	Education level
Smoking habits		
<u>About the father</u>		
Alive/dead	Nationality	Ethnic group
Occupation	Educational level	Smoking habits
<u>About the family</u>		
Residence with the mother	Carer during the day	Co-wives
Haemoptysis in family member *	Number of siblings alive	Number of siblings
<u>Asthma</u>		
Mother *	Father *	Sibling *
<u>Cooking</u>		
Frequency of cooking by mother *	Carriage of the child on the back while cooking *	Carriage of the child on the back while cooking when the child is sick *
Place where the cooking is normally done?		
<u>Nutrition</u>		
Breast fed *		
<u>Other food</u>		
Rice *	Pap *	Cow's milk *
Milk powder or tinned milk *	Fish or meat *	Eggs *
Vegetables *		
<u>Social environment</u>		
Number of people living in same house	Children under 2 years living in same house	Adults sleeping in the same room with the child
Children sleeping in the same room with the child	Children sleeping in the same bed	Children <5 sleeping in same bed
Children <1, <3, <5 years living on the compound (boys and girls separate) *		
<u>About the compound</u>		
Electricity	Water source	Toilet
Walls of house	Roof of house	Mosquito net
Number of rooms in the house	Disposal of rubbish	
<u>Animals on the compound</u>		
Chicken *	Goats *	Sheep *
Cat *	Dog *	
<u>Health care</u>		
Antenatal clinic attendance	Place of delivery	Distance to nearest health facility
Mode of travel to the health facility	Consultation when a child was last sick	

* questions asked in the extended questionnaire in year 2 of the study

3.2.4 Follow-up cohort study

Children with RSV infection admitted to hospital were identified as described above. Children were eligible for inclusion in the follow up cohort if they were under one year of age, lived on the south bank of the Gambia river up to a distance of approximately 40 km away from Banjul, and if the parents agreed to participate. Children for the control cohorts were enrolled from the same village or suburb as the cases. Children in control cohort 1, matched for age with cases, were enrolled after the RSV season had ended and, as a condition for being eligible, had not been admitted to hospital with an ARI during the RSV outbreak. Children in control cohort 1 might therefore have been infected with RSV, but the course of infection was not severe enough to warrant admission. Children in control cohort 2 were from a neighbouring compound of the case, but were born after the RSV outbreak. Children in control cohort 2 were therefore younger than cases and children in control cohort 1, but they were most unlikely to have been infected in early infancy with RSV. Enrolment was undertaken in early 1994, and again in early 1995.

Families of controls were asked to bring their children to MRC for recruitment to ensure that they knew the way to the hospital. A sticker identifying children as study subjects was placed on their health card and their case notes. Mothers were asked to bring their children to MRC if they developed any clinical problem. A field worker was trained to identify study children at the morning outpatient clinic of each working day. After weekends, the inpatient ward was checked for study children admitted over the weekend. Children who presented to the outpatients or who were admitted were seen by a physician who was

unaware of their previous RSV status. Patient notes and health card, which might have contained references to the previous RSV infection were only made available to him after he had filled in the documentation sheet. A standard form containing symptoms and signs relating to the acute presentation was filled in on each attendance of a study child. Respiratory syndromes were defined as follows: pneumonia - lower respiratory tract infection with fast breathing and crepitations (crackles) on auscultation without wheezing; wheezing: ALRI with wheezing, comprising wheezy bronchitis and bronchiolitis; ALRI: pneumonia or wheezing. Children received medical care according to best medical practice in the Gambia, and were admitted for inpatient treatment if this was required. To ensure free access to the MRC hospital, the transport fare was refunded to the patient. If a patient had not come for clinical attendance for more than 6 months, a field worker visited the home to find out whether the study child was still alive, whether the child had been admitted to another hospital in the meantime, or whether the child had moved permanently out of the area. The study was closed for the purpose of this analysis on 31/12/96. In this way, child months at risk could be calculated between enrolment of the child and the end of the study or to the time a child had moved away permanently.

3.2.5 Laboratory methods

3.2.5.1 Immunofluorescence

After obtaining aliquots for viral culture, nasopharyngeal aspirate specimens were homogenised and centrifuged at 350g for 10 minutes. The pellet was then washed in phosphate buffered saline, centrifuged and spread on a microscopy

slide, fixed and stained with a monoclonal, fluorescent conjugated anti-RSV antibody (Dako Diagnostics Ltd., Ely, UK). Slides were read under a fluorescent microscope, and RSV infected cells were identified by their characteristic pattern of fluorescence. Positive controls were included with each batch. Cases were defined on the basis of IF microscopy only.

3.2.5.2 Viral culture, subtyping and sequencing

To obtain viral isolates, a limited number of positive samples were cultured. Samples positive on IF were sequentially inoculated into HEp-2 cells and observed for up to 14 days. If a cytopathic effect developed, RSV was confirmed by IF again, and, if positive, the sample was snap-frozen in liquid nitrogen and stored at -70°C. Up to 30 culture-positive isolates were obtained per season. The cultured isolates were transported in batches on dry ice to the UK, where the isolated virus was subtyped and selected isolates further sequenced (Dr. Pat Cane, University of Warwick, Coventry). The methods used for typing are based on RT-PCR.⁴¹ Briefly, isolates were grouped and further genotyped by restriction analysis of PCR products derived from parts of the nucleocapsid (N) and G genes, together with some nucleotide sequencing of the G gene. Nucleotides 857-1135 of the N gene were amplified and the PCR products digested with *HindIII*, *PstI*, *BglII*, *HaeIII* and *RsaI*. Nucleotides 1-584 of the G gene of group A isolates and 153-817 of group B isolates were also amplified and these products digested with *AluI*, *TaqI*, *MboI* and *MseI*. Nucleotides 285-514 of selected group A isolates were sequenced by automatic sequencing of PCR products.

3.2.5.3 Neutralisation assay

Serum samples were assayed for neutralising antibodies against RSV using a microneutralisation assay against RSV group A (Tracy strain) and B (strain 18537).²⁰¹ Briefly, heat inactivated sera were diluted serially in 96 well tissue culture plates starting at a 3 log₂ dilution. Equal volumes (50 µl) of RSV (+/- 100 TCID₅₀) were added to each well. Positive control wells contained media rather than serum and negative control wells contained media only. These mixtures were incubated at 35°C for 1 hour. After incubation, 100 µl of trypsinised Hep-2 cells (2-4 x 10⁴ cells) in EMEM with 10% FBS were added to each well. The sealed plates were maintained at 35°C in a 5% CO₂ atmosphere. Twenty four hours after the positive control wells showed 100% cytopathic effect (CPE), the cells in all wells were fixed with 10% formalin and stained with 1% crystal violet. The neutralising antibody titre was defined as the final serum dilution at which a >50% reduction in CPE occurred. Reference sera were run with each batch for standardisation and paired samples were run in the same batch.

3.2.6 Statistical methods

3.2.6.1 General

Frequencies were compared using Chi square test or Fisher's exact test, as appropriate. Normally distributed continuous variables were compared using Student's t-test while those which were not normally distributed were compared using the Wilcoxon rank sum test. Analyses were performed using SPSS for Windows, Stata, and EpiInfo software packages.

3.2.6.2 Clinical study and epidemiology

When applicable, variables were adjusted for age before comparison. Multiple logistic regression was used to identify variables correlated with a need for oxygen.

For the estimation of adjusted incidence rates of ALRI, RSV infection, and hypoxaemic RSV infection, for each disease category, a Poisson regression model was developed for the incidence of hospital admission in each settlement including the factors age group, sex, settlement population size, and settlement category (urban or rural according to the 1993 Census). In order to incorporate differences in access to the three hospitals into the model, the cost of travel to the nearest hospital (one way transport fares by the usual public transport - bus, boat, or taxi) was estimated for each village (see 3.2.1.4 and Figure 15).

3.2.6.3 Analysis of risk factor study

Cases and controls were compared by matched analysis to produce estimates of the odds ratio for each variable. Conditional logistic regression was used to calculate odds ratios and 95% confidence intervals adjusted for the effects of other variables. All variables were candidates for inclusion in the regression models but variables were excluded if the odds ratio was not significantly different from 1 at the 10% level and if removing the variable from the model did not alter odds ratios for other variables. Exclusion of variables was done in two phases in order to reduce the effect of missing values on the number of matched sets included in the regressions. First, regressions were done separately for groups of related variables (e.g. nutritional variables, variables related to the family, the social environment - see table 6). Variables which remained in these

models were candidates for inclusion in the overall final model. Lastly each variable was again tested for inclusion in the final model.

This analysis was done first using only variables in the original questionnaire, obtained during the whole study period. In a second step, the additional variables in the extended questionnaire, obtained only after the first year of the study, were also included in the analyses. This led to a second model which explored more variables but which, because of the smaller number of matched sets, had less power. The study had at least 80% power to detect an odds ratio of 2 if the variable occurred with a frequency of 10% in the controls.

3.2.6.4 Follow-up cohort

Forms for each attendance of a study child were entered into a data base. The code of group allocation was broken only after the data entry had been done. Primary endpoints were all episodes of acute lower respiratory tract infection, episodes of ALRI with wheezing, and the number of admissions due to ALRI. A Poisson regression model was developed to determine the incidence rate ratio between case and control groups for each disease category. This model included the factors age, sex, season (rainy or dry season), and care seeking behaviour. Care seeking behaviour was quantified by counting the number of attendances of each child at the clinic for conditions other than respiratory infections.

3.2.7 Ethical issues

The studies were approved by the Gambian Government/Medical Research Council Laboratories Ethical Committee. Verbal consent was obtained from the

guardian for all procedures performed. All treatments given corresponded to the best medical care available in The Gambia.

Chapter 4. RSV in The Gambia

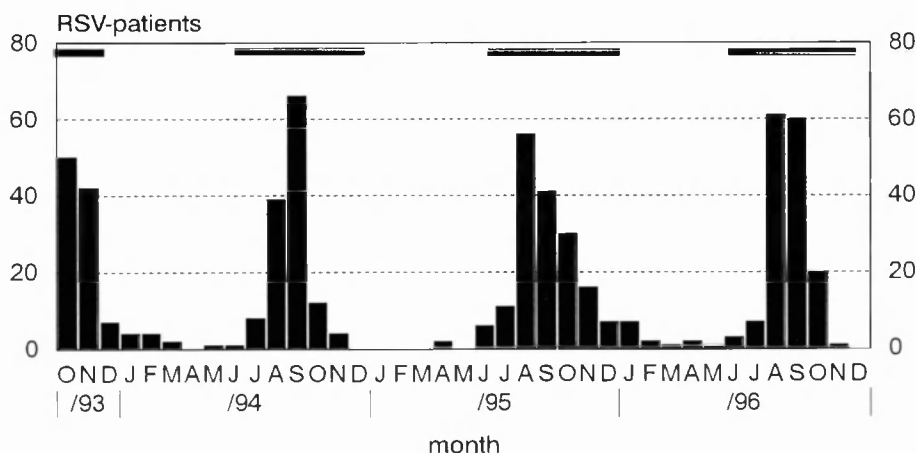
4.1 Epidemiology

4.1.1 Hospital based study

4.1.1.1 Overall description of study patients

During the study period from October 1993 to December 1996, 574 children with RSV infection were identified, 254 at RVH, 210 at MRC and 110 at Sibanor Hospital. Five hundred and eleven children (89%) were admitted for inpatient treatment: 235 to RVH, 181 to MRC, and 95 to Sibanor Hospital. Most of the cases identified presented during outbreaks in the rainy season (July to November) of each year. The number of patients recruited per month is shown in Fig. 16.

Figure 16: Monthly number of RSV cases seen at the three study hospitals over the study period October 1993 to December 1996.

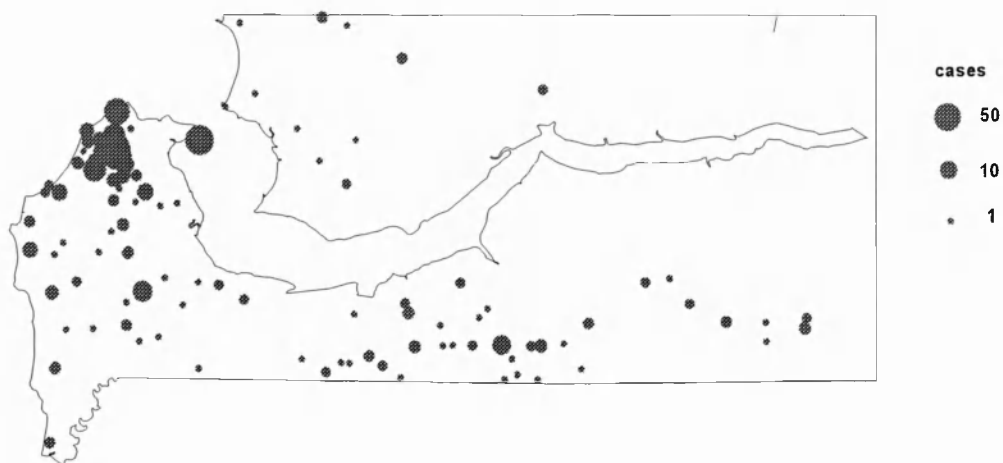


Note: The rainy season is indicated by solid bars over the top

Five hundred and thirty-seven children came from the western region, their geographic origin is shown in Figure 17.

Figure 17: a. Geographic origin of RSV cases at RVH, MRC and Sibamor hospitals from 1993 to 1996 resident in the western region. b. Settlement size and distribution in the western region according to the 1993 census.

a.



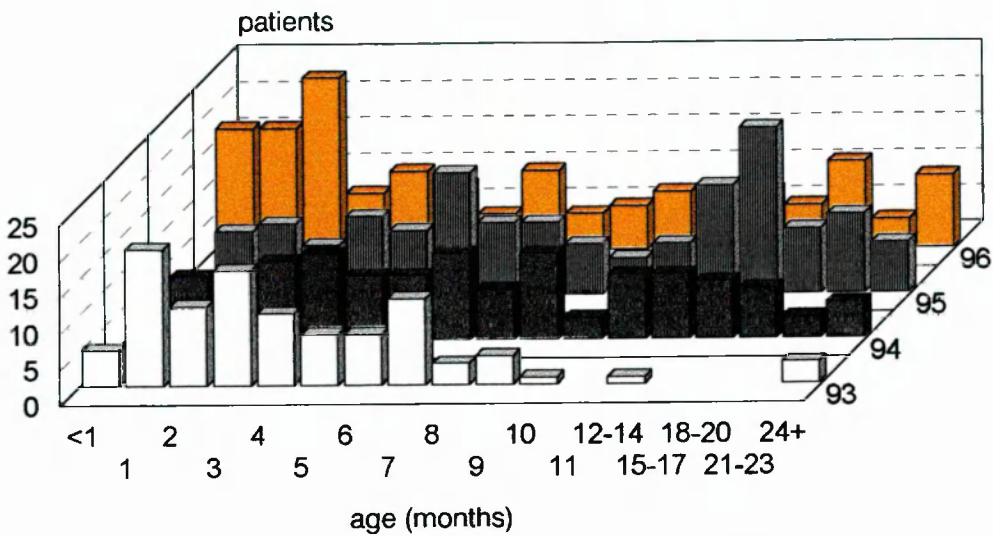
b.



Note: The number of cases from a village (in panel a) or the size of the village (in panel b) is indicated by the size of the dot.

The median age of all children was 6 months, with a range of 9 days to 44 months. The median age of children studied as outpatients (7 months) was little different from those seen as inpatients ($p=0.1$). Age distributions during the 4 outbreaks are shown in Fig. 18.

Figure 1: Age distribution of the children seen during the four RSV outbreaks from 1993 to 1996..



Children were significantly younger in 1993 and 1996 than in the other 2 outbreaks, the median age (interquartile range) being 3 months (2,6) in 1993, 7 months (3,11) in 1994, 8 months (5,15) in 1995, and 5 months (2,11) in 1996 ($p<0.0001$ comparing all outbreaks).

Fifty-six percent of the patients were boys. The sex distribution varied significantly with age: less than 6 months of age, 62% of patients were boys, whereas above 6 months of age, the sex distribution was more equal with 51%

boys ($p=0.008$). Accordingly, the median age of boys was 5 months, while the median age of the girls was 7 months ($p=0.045$).

4.1.2 RSV subtyping

Sixty-seven RSV isolates were further subtyped: 45 were subgroup A and 22 subgroup B. The proportion of infection caused by virus of subgroup A was 90% in 1993, 25% in 1994, 75% in 1995, and 95% in 1996.

4.1.3 Incidence rates

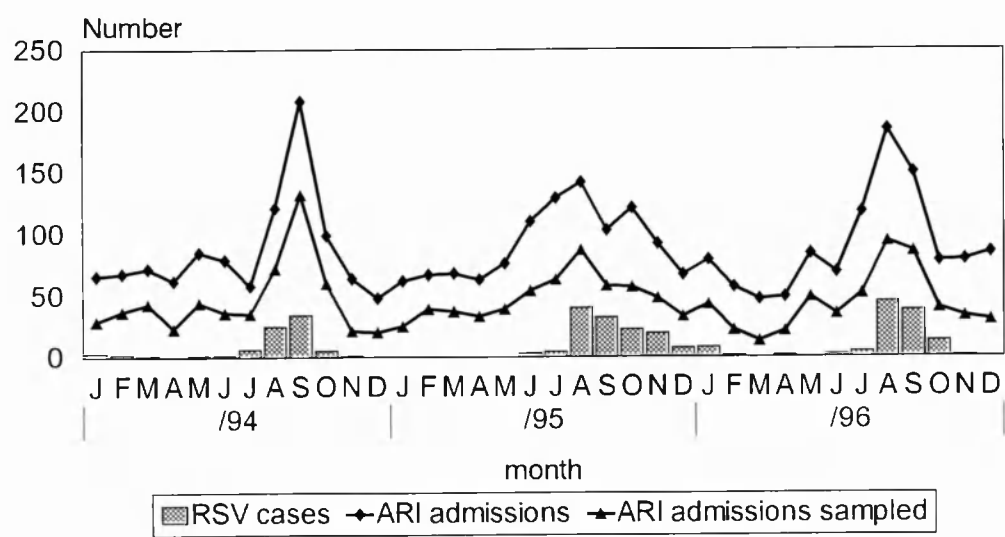
The calculation of incidence rates was restricted to the full study years of 1994 to 1996. During these three years, 3731 children under 2 years of age with a diagnosis of ALRI were admitted to one of the three study hospitals. Thirty-eight had no village of origin documented, 333 came from outside the western region, and 3 had no sex documented. The characteristics of the remaining 3357 children by age group, sex, hospital and year are shown in Table 7.

Table 7: Number of hospital admissions with ALRI, RSV-ALRI, and RSV-ALRI requiring oxygen by age, sex, and hospital from 1994 to 1996.

Age group	year	All ALRI						RSV						RSV with hypoxaemia					
		MRC			RVH			Sibanor			MRC			RVH			Sibanor		
		F		M	F		M	F		M	F		M	F		M	F		M
<1 year	94	124	135	111	148	75	91	15	12	8	28	8	9	1	0	0	6	4	2
	95	123	136	118	152	53	94	19	15	21	29	6	6	2	1	4	5	0	1
	96	99	109	106	137	91	126	18	23	19	25	9	14	3	4	1	5	2	0
1-2 years	94	74	90	63	57	51	54	4	3	3	2	3	1	0	0	0	1	0	0
	95	81	99	75	100	48	85	8	15	9	10	3	5	0	3	1	3	0	0
	96	65	101	62	94	50	80	6	6	2	6	2	3	0	0	0	0	1	0

Nasopharyngeal samples were obtained from 1643 children (49%). During that period, 375 children < 2 years of age from the study area were admitted with proven RSV infection. However, only 319 of these were accounted for with a diagnosis of ALRI in the discharge books of the hospitals and are therefore a subgroup of the above ALRI admissions (23 were not found in the books, 33 had another discharge diagnosis). Figure 19 shows the monthly number of ALRI admissions, the number of children sampled by nasopharyngeal aspirate, and the number of positives.

Figure 19: Monthly number of ALRI admissions, number of children sampled by nasopharyngeal aspirate, and the number positive for RSV in children < 2 years of age resident in the western region of The Gambia.

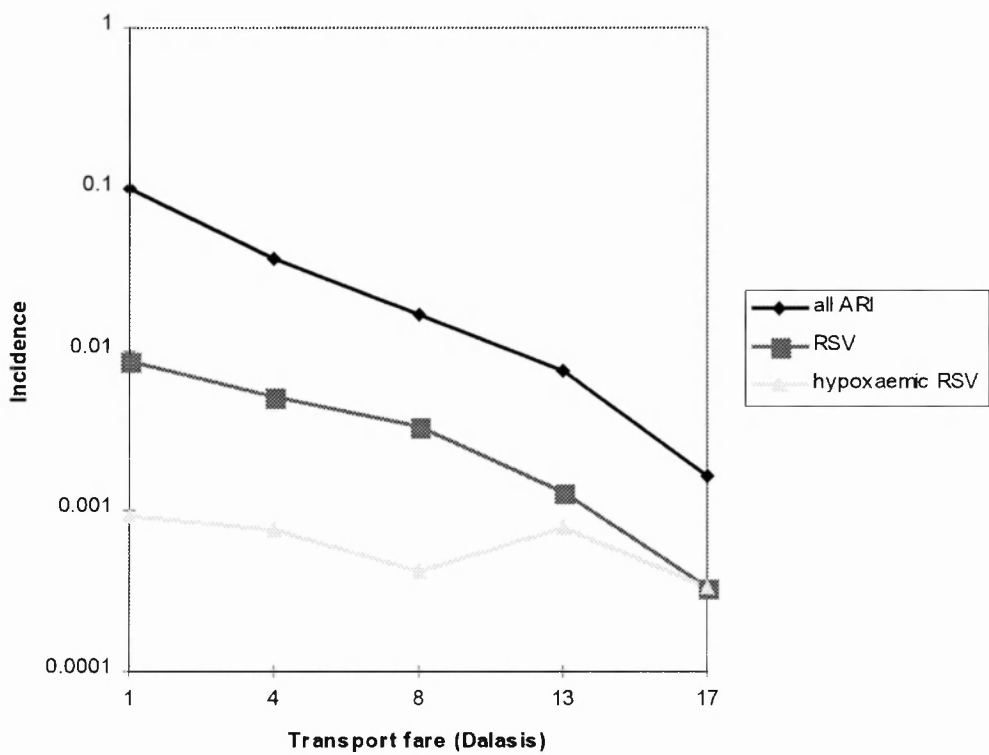


Note: Only RSV cases documented with a discharge diagnosis of ALRI in the hospital routine admission books are included in this graph.

Fifty children with RSV-ALRI were hypoxaemic, characteristics of these children are included in Table 7.

The incidence rates for all ALRI, RSV-ALRI, and RSV infection with hypoxaemia varied with distance from the hospital as shown in Figure 20.

Figure 20: Incidence rate of all ALRI admissions, admission with RSV-ALRI, and hypoxaemic RSV infection in children under 1 year of age by transport fare (public transport) to hospital.



Note: Transport fares are grouped into 0-2 Dalasis (1), 3-5 Dalasis (4), 6-10 Dalasis (8), 11- 15 Dalasis (13), and above 15 Dalasis (17) (10 Dalasis = 1 US\$).

The incidence rate was highest in children nearest to hospital. For those whose transport fare to hospital was 2 Dalasis or under (10 Dalasis = 1 US\$), the observed incidence rates for children under 1 year of age with ALRI was 10 per hundred children per year. For admission with RSV-ALRI, the incidence was 0.9 per hundred children per year, and for hypoxaemic RSV-ALRI, 0.1.

Incidence rate for ALRI was significantly higher in males, in younger children, in rural settlements, and decreased as the size of settlement increased. For RSV-ALRI, incidence was also higher in males and in younger children, and decreased with increasing settlement size, but the difference between urban and rural settlements was not significant. In the case of hypoxaemic RSV-ALRI, incidence varied with age but was not associated significantly with sex, settlement size, settlement type, or travel cost to hospital. Incidence did not vary significantly year to year for any of the diseases. Incidence rate ratios are shown in table 8.

Table 8: Adjusted incidence rate ratios (IRR) for different categories of variables influencing the incidence of ALRI, severe RSV infection leading to hospital admission and hypoxaemic RSV infection.

Variables	categories	all ALRI		RSV-ALRI		hypoxaemic RSV-ALRI	
		IRR	95%CI	IRR	95%CI	IRR	95%CI
Transport cost	per Dalasi increase	0.75	0.74-0.77	0.85	0.81-0.89	0.91	0.82-1.0
Sex	female	1		1		1	
	male	1.25	1.17-1.34	1.27	1.03-1.56	1.54	0.88-2.73
Age	0-11 months	1		1		1	
	12-23 months	0.55	0.51-0.59	0.32	0.25-0.40	0.20	0.09-0.42
Village size	Increase in settlement	0.84	0.82-0.86	0.81	0.75-0.87	0.87	0.72-1.04
	population size of 2500						
Settlement type	Rural	1		1			
	Urban	0.71	0.64-0.78	1.27	0.94-1.71	1.12	0.49-2.55

The incidence of ALRI in children under 1 year of age, corresponding to travel cost of 2 Dalasis or less, estimated from the regression model, was 11.4 (95%CI 10.6,12.3) per hundred children per year for boys in urban areas, and 9.1 (8.4,9.9) for girls, and in rural areas 16.1 (14.6,17.6) for boys and 12.8 (11.6,14.1) for girls. For RSV-ALRI, the incidence was 1.3 (1.1,1.6) per hundred children per year for boys, and 1.0 (0.8,1.3) for girls, in both urban and rural areas. For hypoxaemic RSV-ALRI, the corresponding estimates were 0.1 (0.07,0.19) and 0.07 (0.04,0.13) for boys and girls respectively.

19.4% (319/1643) of ALRI cases who were sampled were positive for RSV, but only 49% of ALRI cases had nasopharyngeal aspirates taken. Thus the RSV incidence figures underestimate the true incidence, which may be twice as high (2.6% for boys, 2% for girls).

4.1.4 Household study

During the 1993 and 1994 RSV seasons, 103 case- and 22 control compounds were visited. The mean number of children in the case and the control compounds were 5.2 (SD 3.5) and 5.1 (SD 3.0), respectively. Paired serum samples were obtained in 68 case compounds and 22 control compounds from 237 case compound and 83 control compound children 5 years of age and below. The mean age of these children was 29 months (SD 17) for both the case- and the control compounds. Of the 320 children studied, 85 (27%) had either a rising antibody titre or virus detected by IF; 25% of the children in the case compounds and 31% of children in the control compounds. The rate of infection in the individual compounds ranged from 0% to 100%. Infection rates were lowest in

the 2-3 years old (16%) and equally high in the 0-1 and 4-5 years olds (33%). Incidences of infection did not differ significantly between case and control compounds and between age groups.

4.1.5 Discussion

RSV disease has been studied in Gambian children during four rainy season outbreaks. During these outbreaks, RSV was a major cause of hospital admission. It was shown that distance to hospital influenced admission for ALRI overall and for RSV-ALRI overall. Comfortingly, and surprising, a significant decline of cases with distance from hospital was not found for RSV-ALRI cases with hypoxaemia. As the three study hospitals are the only ones in the western region that provide oxygen, this finding indicates that the referral system worked well for the most severe cases.

The incidence rate for ALRI admission to hospital in the first year of life and the villages nearest to the health facilities of around 10% is on the lower side of that reported in other studies^{30,32,75,146,247,252}, but the children in our study represented only the more severe end of the spectrum of ALRI.

The incidence rate of RSV-ALRI observed is likely to be a gross underestimate. Only half the children with ALRI were sampled, and in those sampled we are likely to have missed an additional number of cases, as diagnosis with immunofluorescence alone has a limited sensitivity,^{114,218} and the transport of samples from distant health facilities further decreased the yield. Estimating from the excess of admissions with ALRI during the RSV season, we estimate the real incidence to be around 3% for admissions with RSV-ALRI during the first year of life. This is a similar incidence for RSV-ALRI to that reported from

Alaskan Natives ²²⁸, but higher than that reported in Bedouins from southern Israel ⁵⁷. We found a higher incidence rate in boys than in girls, an almost universal finding in ALRI. The difference in the observed incidences with distance might indicate, however, that part of this difference is due to better care seeking behaviour for boys. Incidence rates for ALRI were higher in rural settings, but for RSV-ALRI, the incidence rates were higher for urban areas. For both ALRI and RSV-ALRI, the incidence rates were lower in bigger villages. As most of the smaller villages are poorer than the bigger ones, this might indicate the influence of the overall living standard on the incidence of ALRI, but to a lesser degree on severe RSV infection. A steady decline in the incidence rate ratio for the second year of life from 0.55 for all ALRI, to 0.32 for RSV-ALRI and 0.2 for hypoxaemic RSV infection was observed (Table 8), indicating that RSV plays less of a role in the second year of life than other pathogens, and that hypoxaemia becomes uncommon.

These outbreaks suggest a predilection of RSV for the wet season, as has been described from other tropical countries with seasonal rain fall. ^{42,50,109,185,237,241}

Although only a limited number of viral isolates was subtyped, we observed some alternation between the predominant subgroups of virus. This alternating pattern, and the overall dominance of subgroup A, are similar to the situation described in temperate climates. ^{40,78,104,112,210,246}

Children in 1993 were significantly younger than those seen in the other years.

This is likely to be due in part to the study design, as children who were enrolled in the *Haemophilus* vaccine trial were more likely to have been investigated as

possible cases of RSV infection than other children, and in 1993, the vaccine trial had just started, so only young children had been enrolled in the trial. In addition, the study started in the middle of an outbreak, and, as noticed in the other three years, the mean age of children decreased as the outbreak progressed. A further possible explanation for the change in the age distribution observed might be limited cross immunity between viral subgroups. In general, the age distribution observed is consistent with that reported from other developing countries, where, on average, half the children in hospital based studies were reported to be less than 6 months of age.^{49,54,66,67,76,193,238} The majority of the children less than 6 months of age were boys, as described in developed countries,⁸⁶ possibly because of smaller airways in boys.

In the compound study, we were able to demonstrate that, during outbreaks of RSV, the virus circulates widely in the community. Between a quarter and a third of children in the compounds investigated had evidence of recent infection. This figure is likely to be an underestimate, as we visited compounds only after an index case, usually a young infant, had been admitted to hospital. If the number of new cases in the compound follows a bell shaped distribution, we are likely to have visited the compound when the number of new cases was already declining. Thus it is possible that the true number of infected children on the compounds was double the number that we observed. In community based studies in industrialised countries, infection rates of between 69%⁸⁹ and 98%¹¹¹ have been observed in the first year of life, with little¹¹¹ or no decline⁸⁹ in the second year of life. Monto¹⁶⁷ found a yearly infection rate in the age group of 5-

9 years olds of 19.7 %. In a family study, Hall reported an overall infection rate of 29% for infants, and of 63% within infected families.

In summary, we have shown that RSV was responsible for a considerable number of hospital admissions in small children in the Gambia, especially during the rainy season. It poses a considerable burden on the health system through a demand on hospital beds and oxygen. Thus, prevention of RSV infection by vaccination is likely to be cost effective.

4.2 Clinical spectrum of RSV infection

4.2.1 Presenting complaints and physical signs

In 519 of the 574 (90%) children with RSV infection, clinical information was available to analyse presenting complaints and physical signs during presentation to hospital. The main presenting complaints were cough (87%), fever (84%), “chest pain” (25%), vomiting (22%), breathing difficulty (21%), diarrhoea (10%), fast breathing (9%) and poor feeding (3%). The median duration of the main symptom was 3 days. Physical findings on examination were available from 472 inpatients and 47 outpatients; the main physical signs, and differences in signs between in- and outpatients, are presented in Table 9.

Table 9: Comparison of physical findings in inpatients and outpatients with RSV disease. (N=519, percents in brackets)

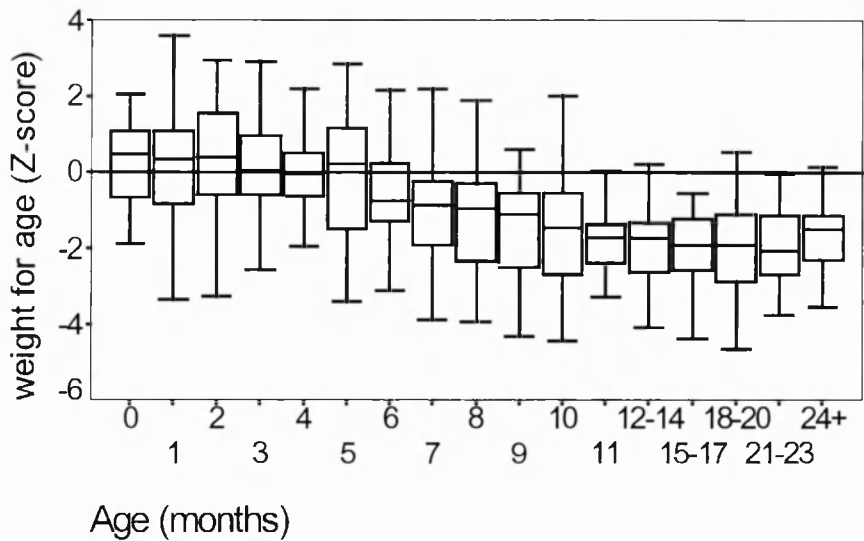
Sign	Categories	Inpatients (n=472)	Outpatients (n=47)	p-value
nasal flaring	present	191 (40%)	22 (47%)	0.47
lower chest wall indrawing	moderate or severe	241 (51%)	16 (34%)	0.03
grunting	present	130 (28%)	9 (19%)	0.28
crepitations	present	379 (80%)	19 (40%)	<0.0001
wheezing	present	182 (39%)	35 (75%)	<0.0001
rhonchi	present	163 (35%)	30 (64%)	0.0001
wheezes <u>or</u> rhonchi	present	240 (47%)	40 (85%)	0.0001
respiratory rate	mean (SD)	65.7 (14.9)	55.6 (11.2)	<0.0001
temperature	mean (SD)	38.1 (0.96)	38.1 (0.86)	0.6
SaO2	median (25%,75%)	95 (92,98)	98 (96,99)	<0.0001
heart rate	mean (SD)	157 (18.4)	153 (18.9)	0.22

Clinical examination of the chest was normal in 41 children (8%). Young children had a lower average temperature than older ones: the median (interquartile range) temperature of children less than 3 months was 37.6°C (37.1,38.2), that of those between 3 and 5 months 38.1°C (37.6,38.7), and that of those aged 6 months to 23 months of age was 38.4°C (37.8,39.1). Median temperature dropped again for those 2 years of age and above to 37.6°C (37.1, 38.0) ($p<0.0001$ for overall comparison between all groups).

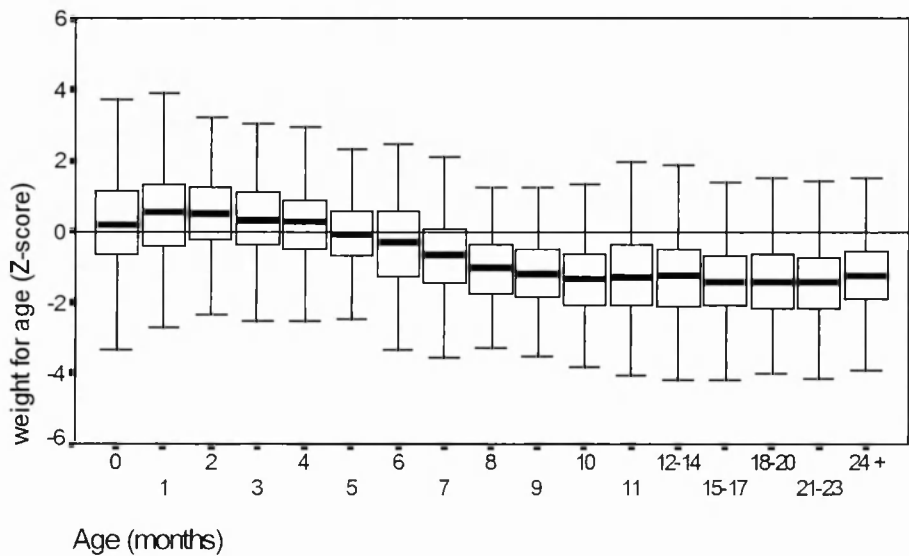
Up to 6 months of age, RSV cases were above the mean weight of the NCHS standard,⁶³ after 6 months of age, the weight for age dropped gradually against the NCHS standard (Fig. 21).

Figure 21: a. Weight for age of children with RSV infection by age. b. Weight for age of presumably healthy children from several surveys in The Gambia¹⁵²

a



b



Note: Ages are in months up to one year, then in 3 monthly groups up to 2 years, and then grouped together above. The box plot depicts the interquartile range as a box, and the median as a line in the box. The bars depict the maximum and minimum values.

Presenting diagnoses were pneumonia in 74% of cases, bronchiolitis in 40%, and asthma in 0.8%. Ninety-seven percent of children had a diagnosis of pneumonia or bronchiolitis. Additional diagnoses were malaria (4.0%), congenital heart disease (1.6%), severe malnutrition (2.6%), and sickle cell disease (0.4%).

Thirteen (2.4%) children had underlying conditions likely to increase the risk of severe RSV disease (8 congenital heart disease, 2 Down's syndrome, 1 rickets, 1 malformed lung, 1 hydrocephalus). The median duration of hospital stay was 3 days, with a range from 1 to 28 days (interquartile range: 2,5 days). Thirteen children (2.4 %) died. Details of these children are presented in Table10. All children who died received antibiotics, most of them chloramphenicol or ampicillin and gentamycin.

Table 10: Characteristics of 13 children who died with an RSV infection. All observations are on admission to hospital.

no	age (months)	sex	temp (°C)	SaO2	weight [weight for age] (Z-score)	comments	duration between		death probably RSV-related
							admission and	death	
1	42	M	39.0	92%	12 kg [-1.93]	cerebral malaria, RSV positive on day 3 after admission.		4 days	no
2	17	F	36.6	not done	5.2 kg [-4.71]	marasmic kwashiorkor. RSV positive on day 1.		16 days	no
3	1	M	not done	not done	3.4 kg [-1.3]	sepsis, died unexpectedly.		24 hours	yes
4	4	M	39.2	96%	9.0 kg [2.53]	Temperature 41 °C, hypoglycaemic in night.		24 hours	yes
5	4	M	38.4	not done	6.2 kg [-0.49]	severe dehydration, recurrent		36 hours	yes
6	16	M	37.8	not done	6.2 kg [-4.37]	malnutrition, chest problems, deteriorated continuously.		4 days	yes
7	22	F	36.5	not done	7 kg [-3.74]	no details of death		24 hours	yes
8	12	M	39.2	not done	6 kg [-4.09]	pneumonia with heart failure		18 hours	yes
9	6	M	35.7	not done	7 kg [-0.87]	pneumonia, hypothermic, unconscious.		20 hours	yes
10	2	F	38.1	not done	2.1 kg [-3.83]	congenital heart disease, failure to thrive		3 days	yes
11	2	M	37.4	95%	4.7 kg [-0.57]	improving initially, died unexpectedly		2 days	yes
12	3 weeks	M	36.4	84%	1.6 kg [-4.07]	neonatal sepsis, failure to thrive, recovered from acute illness, cause of death unclear		12 days	no
13	3	F	39.1	86%	3.6 kg [-1.63]	febrile and hypoxaemic throughout, progressive deterioration		3 days	yes

4.2.2 Oxygen

Eighty of the 511 children admitted to hospital (16%) received oxygen because of hypoxaemia, defined as an arterial haemoglobin oxygen saturation below 90%, or because this was warranted on clinical grounds in patients whose saturation was not documented. The median duration of stay of children who received oxygen was 5 days. The median age (interquartile range) of children who required oxygen was 4 months (1,8), compared with a median age of 6.5 months (3,12) for children who did not require oxygen ($p=0.002$). Forty percent of admitted children younger than 1 month, and 24% of those between one and two months of age required oxygen. At the other end of the age spectrum, 19% of children older than 18 months required oxygen, whereas in those between these age groups, 12% required oxygen.

4.2.3 Predictors of hypoxaemia

In a multiple logistic regression model, factors associated with hypoxaemia were age, temperature and respiratory rate. Children were more likely to require oxygen if they were very young or older than 18 months, the risk was lowest for children of intermediate ages (see univariate analysis above). After adjusting for the influence of age (using age as a quadratic term), a drop in temperature (OR 1.4, 95% CI 1.03,1.88 for each degree of temperature) and increase in respiratory rate (OR 1.48, 95% CI 1.25,1.76 for an increase in respiratory rate of 10 breaths per minute) were significantly associated with hypoxaemia

4.2.4 Concomitant bacterial infections

Blood cultures were obtained from 255 children. Nine cultures were positive, seven of which were blood cultures (2.7% of all blood cultures). Four isolates were *S. pneumoniae*, 2 *H. influenzae* type B, 2 *S. aureus*, and one *Enterobacter agglomerans*. All organisms were found in blood cultures, except for one *H. influenzae* which was isolated from a pleural effusion, and one *S. aureus* which was isolated from a lung aspirate. The mean temperature of the bacterial culture positive cases was significantly higher than the mean temperature of those children who were bacterial culture negative (39.2°C vs. 38.2°C, $p=0.01$). No other physical findings were significantly different between bacterial culture positive and bacterial culture negative cases.

4.2.5 RSV subtyping

The duration of stay in hospital was significantly longer for children with subgroup B infection as opposed to subgroup A (median 5 days versus 3 days, $p=0.02$). No other clinical measurement differed significantly between the two RSV subgroups.

4.2.6 Discussion

The history of most children was very short. Presenting complaints are specific to the cultural setting; fever is the complaint mentioned by the mothers of almost all sick children in the Gambia, irrespective of temperature. Chest pain denotes any problem conceived as coming from the chest. In contrast to a study in India,⁵⁰ rapid breathing or indrawing was rarely mentioned in The Gambia.

Gastrointestinal symptoms were mentioned in about a quarter of the patients.

The strongest predictors of admission were the presence of crepitations, higher respiratory rate and lower age, similar to findings from developed countries.^{90,175}

In contrast to a study from Australia,¹⁷⁵ the risk of requiring oxygen increased with increasing respiratory rate. The higher frequency of wheezing seen in children studied in outpatient as opposed to those seen in hospital might in part be explained by the study design, i.e. children with wheezing were included in the outpatient study, whereas children with a mild respiratory disease not requiring admission would normally not have been investigated. A low prevalence of wheezing in children with proven RSV disease has been reported from developed countries previously.³¹ The age-dependency of temperature is noteworthy. Younger children rarely responded with an increased temperature, in line with findings from Israel, where only 40% of the children had a temperature above 38°C.⁵⁷ In contrast, all the children with a positive blood culture had a temperature of 38.5 °C or above. Higher temperature being a predictor of positive blood cultures was previously found in another study in the Gambia.²³ This might be a useful guideline to decide which children seen during RSV outbreaks require treatment with antibiotics. Current treatment algorithms for acute respiratory infections do not consider temperature.^{266,267}

The nutritional status of the study children was generally good; children less than 6 months of age were heavier than the American standard. Although weight for age fell in older children in comparison with the NCHS standard, children with RSV infection did not differ from other Gambian children.¹⁵² This is in agreement with a study from Nigeria, where RSV was not found more frequently in malnourished children,¹⁸⁶ and with a previous study from The Gambia, in

which RSV was found significantly less frequently in malnourished than in well nourished children with pneumonia.¹

Thirteen children died. Three of these deaths were probably unrelated to RSV infection, reducing the in hospital death rate to 2% . This relatively low case fatality rate is explained in part by the availability of oxygen in the hospital and by the existence of established guidelines for oxygen therapy. It is noteworthy that no deaths occurred in the first study year, when special efforts were made to identify hypoxaemic children for a concurrent study on methods of oxygen delivery.²⁶⁰ On the other hand, very few children with underlying heart or lung disease were found in the study population. Most of the study children were routinely treated with antibiotics, but no specific anti-viral agents are available in The Gambia.

The low rate of positive blood cultures obtained (2.7%) is in concordance with findings from developed countries, where bacterial superinfection in RSV infection is considered unusual,¹⁰² but contrasts with findings from Pakistan, where 31% of RSV infected children had a positive blood culture.⁸² A previous study in The Gambia, in which only very sick infants were included, found a bacterial organism in 7/32 (22%) of cases of RSV infection.⁷⁶ However, in this early study, the diagnosis of a bacterial infection was based on serology as well as culture and may have overestimated frequency. Most other studies from developing countries report very small numbers of culture positive cases, the isolation rate varying between 2% and 10%.^{1,2,50,115,118,124,248,253} The predominant organisms found in our study, *S. pneumoniae* and *H. influenzae*, were the same as those obtained in these other studies. As blood cultures are insensitive for the

identification of bacterial causes of pneumonia, all these numbers are probably underestimates, but nevertheless the rate of bacterial infection appears to be low in Gambian children with RSV disease.

In summary, the clinical presentation of children with RSV infection is similar to that in developed countries. The frequency of underlying conditions is low, however, indicating that it is mainly otherwise normal children who develop severe RSV infection. Concomitant bacterial infections are uncommon. As the acute mortality observed in those children is not negligible, efforts at prevention and case management with oxygen are worthwhile.

4.3 Risk factors for RSV infection leading to hospital admission: a case control study

Two hundred and seventy-seven cases were enrolled in the study (representing 79% of hospital admissions with RSV infection during that time, with another 4% coming from outside the study area), together with 364 controls. The extended questionnaire was completed for 172 cases and 222 controls. Fifty-five percent of the cases were boys, as were 50% of the controls. The median (interquartile range) age was 9 months (5,14) for cases and 9 months (5,15) for controls.

The statistically significant results of the univariate analysis of the original questionnaire are shown in table 11, the results of the extended questionnaire are shown in table 12.

In the final regression model (Table 11) based on variables in the original questionnaire administered over two years, four main risk factors for severe RSV disease were identified: a larger number of people living in the house; having had a sibling who died; living in a house with a flush toilet rather than pit-latrine or no toilet; and belonging to a family with a non-Gambian, rather than a Gambian, father. There were also slight effects of father's occupation and the type of child care, but these were of borderline significance.

Table 11: Estimated crude and adjusted odds ratios for socio-economic and environmental factors associated with severe RSV infection (cases vs. controls). The comparison is based on 277 matched sets.

Potential risk factor	categories	cases	controls	crude odds ratio	p-value	adjusted odds ratio	p-value
people living in the house toilet	10 or more vs. less than 10	52%	29%	1.59 (1.14, 2.2)	0.006	3.06 (1.92, 4.89)	<0.001
	flush toilet vs. other or no toilet	9.7%	3.6%	3.8 (1.75, 8.23)	0.001	5.54 (2.11, 14.6)	0.001
	1 or more vs. none	38%	23%	2.24 (1.55, 3.23)	<0.001	2.11 (1.33, 3.35)	0.001
	Gambian vs. non Gambian	83%	90%	0.47 (0.29, 0.77)	0.003	0.48 (0.25, 0.91)	0.027
	farmer	24%	21%	1		1	
fathers occupation	manual worker	19%	23%	0.76 (0.43, 1.34)	0.35	0.65 (0.31, 1.36)	0.25
	non-manual professional	48%	43%	1.05 (0.64, 1.73)	0.83	0.99 (0.50, 1.95)	0.98
	army/police	1.6%	6.3%	0.22 (0.07, 0.71)	0.011	0.23 (0.05, 1.02)	0.054
	unemployed	7.7%	5.4%	1.46 (0.63, 3.37)	0.37	1.48 (0.51, 4.25)	0.46
	mother vs. other person	92%	84%	1.66 (0.98, 2.8)	0.06	1.76 (0.91, 3.40)	0.09
child care during the day water source	tap in compound vs. other water sources	21%	17%	1.48 (0.95, 2.34)	0.085	1.75 (0.85, 3.60)	0.12
mothers ethnic group	Mandinka	45%	40%	1		1	
	Wolof	9.4%	11%	0.82 (0.45, 1.45)	0.49	0.5 (0.2, 1.22)	0.13
	Fula	11%	11%	1.02 (0.58, 1.78)	0.94	0.73 (0.31, 1.76)	0.49
	Jola	22%	27%	0.66 (0.41, 1.05)	0.08	0.64 (0.33, 1.24)	0.19
	other	13%	11%	1.09 (0.63, 1.89)	0.74	0.62 (0.26, 1.43)	0.27
children living on the compound	4 or more vs. less than 4	19%	12%	1.7 (1.09, 2.66)	0.02	1.52 (0.81, 2.85)	0.19
electricity in house siblings alive co-wives place of delivery	yes vs. no	32%	37%	0.7 (0.47, 1.04)	0.08	0.73 (0.36, 1.50)	0.40
	3 or more vs. less than 3	48%	40%	1.48 (1.05, 2.09)	0.023	1.17 (0.71, 1.96)	0.53
	1 or more vs. none	42%	29%	1.84 (1.3, 2.6)	0.001	0.88 (0.51, 1.52)	0.65
	home vs. other places	37%	32%	1.37 (0.95, 1.99)	0.087	0.99 (0.58, 1.75)	0.99

Note : Numbers for cases and controls are presented as unmatched percentages, odds ratios are based on matched pairs analysis.

Parental smoking was equally common for cases and controls: 39% of the fathers of cases smoked, compared with 41% of fathers of controls. Mothers smoked infrequently (2% in both groups).

To examine the additional variables in the extended questionnaire, the second step of the analysis was restricted to the second year of the study (Table 12). Variables in the original questionnaire had similar unadjusted odds ratios in the second year and for both years combined, except for child care by the mother which was associated with increased risk in the combined data but with reduced risk in the second year.

Table 12: Estimated crude and adjusted odds ratios for socio-economic, environmental and nutritional factors associated with severe RSV infection (cases vs. controls). The comparison is based on 172 matched sets using the extended questionnaire.

Potential risk factor	categories	cases	controls	crude odds ratio	p-value	adjusted odds ratio	p-value
children between 3 and 5 years of age	2 or more vs. less than 2	37%	14%	3.6 (2.12, 6.1)	<0.001	5.67 (2.80, 11.15)	<0.001
eating vegetables	yes vs. no	22%	36%	0.33 (0.17, 0.64)	0.001	0.18 (0.07, 0.48)	<0.001
siblings dead	1 or more vs. none	34%	19%	2.47 (1.47, 4.11)	0.001	2.72 (1.40, 5.28)	0.003
mother cooking	once daily or more vs. less than once a day	71%	83%	0.48 (0.28, 0.81)	0.007	0.32 (0.15, 0.70)	0.004
fathers nationality	Gambian vs. non Gambian	84%	95%	0.47 (0.29, 0.77)	0.003	0.23(0.08, 0.67)	0.007
fathers occupation	farmer	26%	22%	1		1	
	manual worker	20%	24%	0.62 (0.30, 1.30)	0.21	0.43 (0.16, 1.11)	0.082
	non-manual professional	46%	41%	0.93 (0.49, 1.76)	0.81	0.89 (0.40, 2.01)	0.79
	army/police	1.1%	7.7%	0.11 (0.023, 0.54)	0.006	0.13 (0.02, 0.73)	0.021
water source	unemployed	6.9%	4.7%	1.5 (0.46, 5.08)	0.48	5.1 (0.98, 26.5)	0.052
	tap in compound vs. other water sources	21%	14%	2.02 (1.10, 3.71)	0.023	2.18 (0.88, 5.37)	0.09
electricity in house	yes vs. no	30%	38%	0.58 (0.35, 0.98)	0.042	1.95 (0.42, 8.99)	0.40
children under 5 years on the compound	increase per child			1.9 (1.26, 2.85)	0.002	1.15 (0.82, 1.61)	0.40
toilet	flush toilet vs. other or no toilet	7.2%	2.7%	2.9 (1.02, 8.27)	0.044	2.45 (0.25, 23.42)	0.44
place of delivery	home vs. other places	38%	28%	1.42 (0.91, 2.23)	0.12	1.11 (0.45, 2.70)	0.45
child carried on the back while cooking when the child is sick	yes vs. no	65%	73%	0.58 (0.35, 0.94)	0.028	0.65 (0.19, 2.27)	0.50

Table 12 ct.

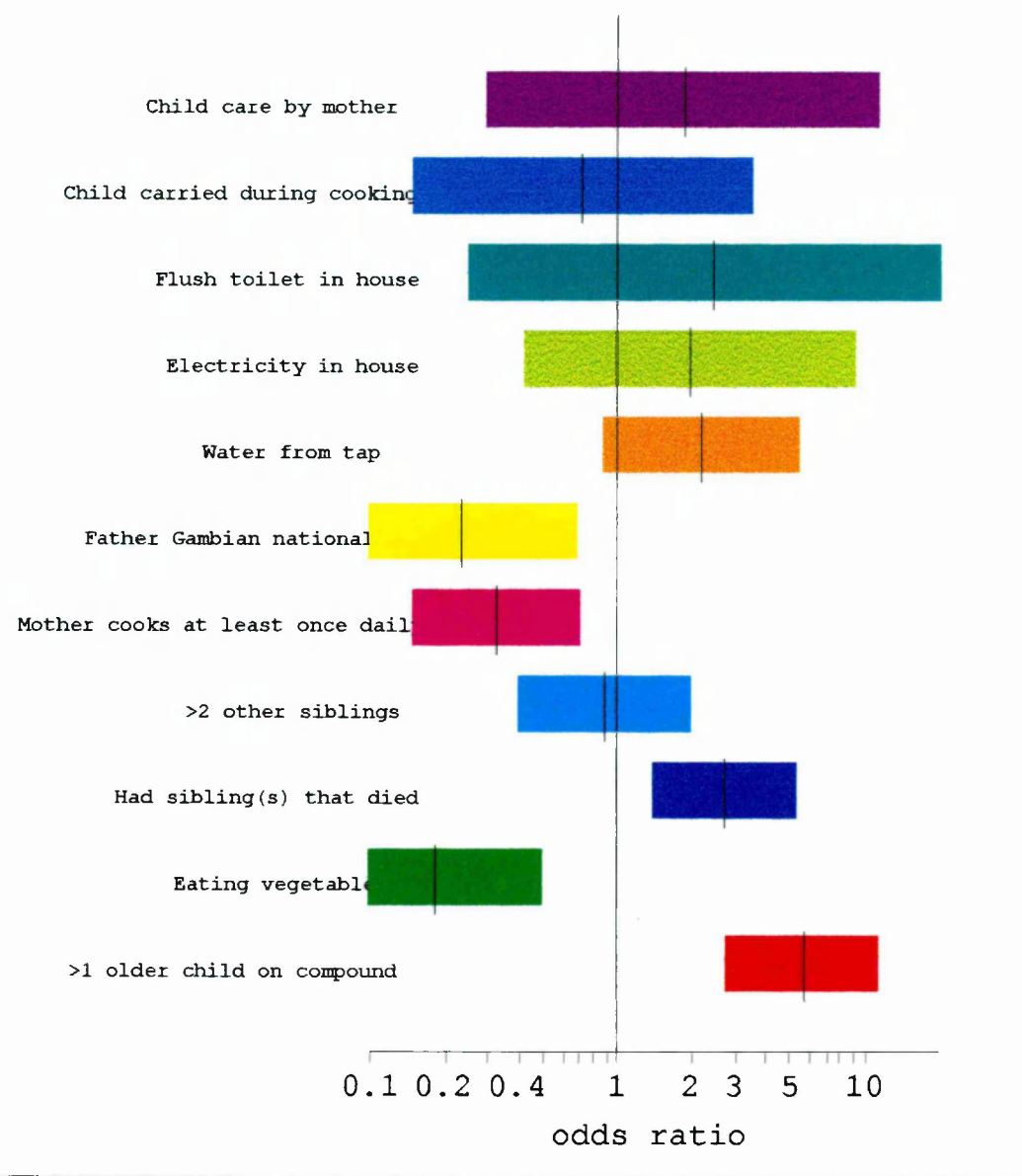
Potential risk factor	categories	cases	controls	crude odds ratio	p-value	adjusted odds ratio	p-value
child care during the day	mother vs. other person	92%	97%	0.35 (0.14, 0.90)	0.029	1.85 (0.30, 11.3)	0.50
children living on the compound	4 or more vs. less than 4	17%	13%	1.54 (0.84, 2.81)	0.16	0.58 (0.10, 3.38)	0.55
people living in the house	10 or more vs. less than 10	47%	42%	1.12 (0.66, 1.9)	0.67	1.28 (0.53, 3.09)	0.58
child carried on the back	yes vs. no	63%	71%	0.6 (0.38, 0.91)	0.018	0.71 (0.15, 3.47)	0.67
while cooking							
eating pap	yes vs. no	75%	81%	0.59 (0.33, 1.01)	0.057	0.83 (0.29, 2.37)	0.73
siblings alive	3 or more vs. less than 3	47%	40%	1.46 (0.94, 2.26)	0.087	0.88 (0.40, 1.95)	0.77
co-wives	1 or more vs. none	41%	28%	1.76 (1.13, 2.72)	0.011	0.91 (0.36, 2.34)	0.85
mother asthma	yes vs. no	4.2%	0.5%	8 (1.0, 64)	0.05	--	*

Numbers for cases and controls are presented as unmatched percentages, odds ratios are based on matched pairs analysis.

*: not included in the final model because an odds ratio could not be calculated due to a 0 in one cell.

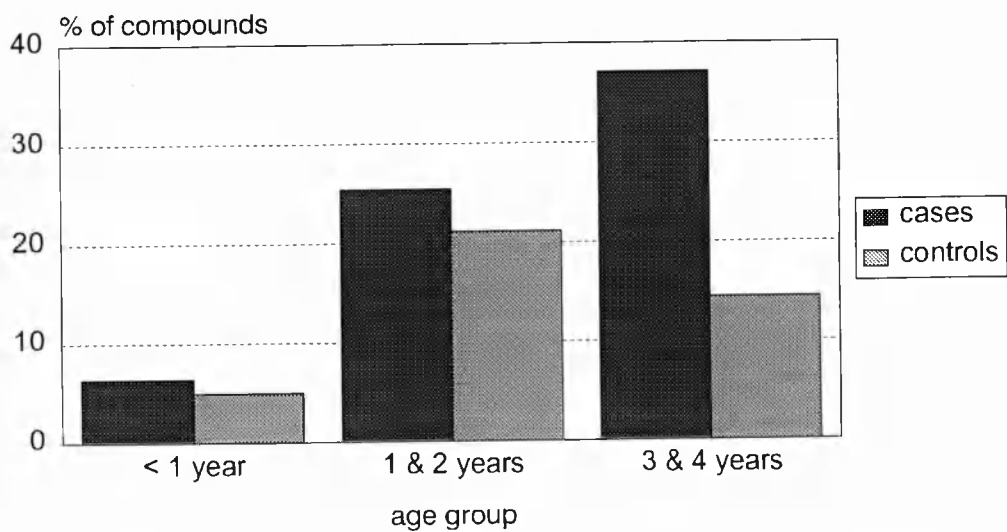
Figure 22 shows selected parameters and their odds ratios from Table 12 in graphic form.

Figure 1: Adjusted odds ratios for statistically significant and other selected risk factors for severe RSV infection based on the extended questionnaire.



There were more detailed questions in the extended questionnaire about numbers and age groups of children in the compound. Some of these new variables were more strongly associated with severe RSV disease than the variables in the original questionnaire. The adjusted odds ratios for those other variables were smaller and lost significance in this second analysis. Examining the contribution of numbers of children of different age groups on the compound, the strongest risk factor was a larger number of children in the 3-5 years age group (Table 12 and Figure 23). The sex of these children had no effect on risk either when numbers of boys and girls or when numbers of children of the same or different sex as the index case were considered.

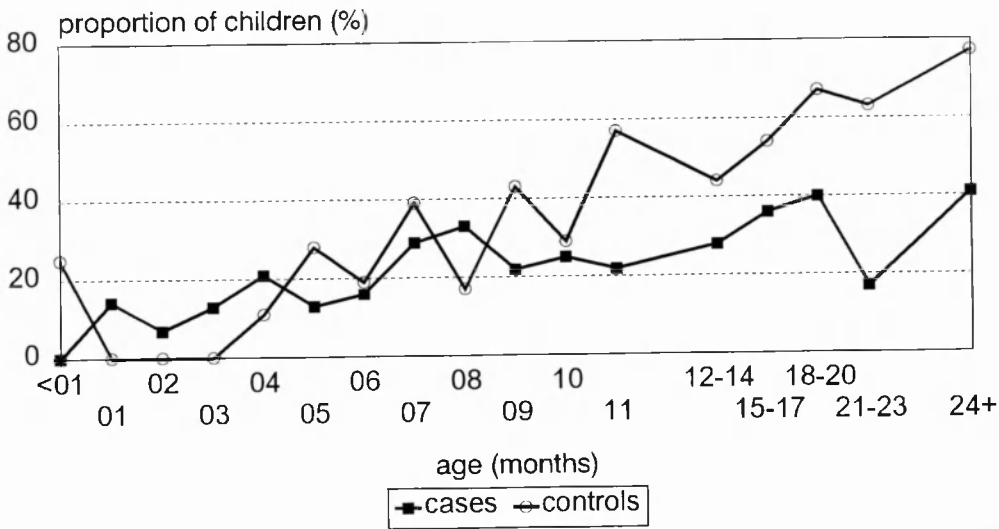
Figure 23: Percentage of compounds with 2 or more children in the age groups under 1 year, aged 1 or 2 years, and aged 3 or 4 years resident on the compound.



The extended questionnaire included new questions on nutrition, asthma in the family, and exposure to cooking fumes. Eating vegetables was more common in

controls than in cases (OR 0.18, 95%CI 0.07, 0.48) , the difference was found to be more marked in older children (Figure 24).

Figure 24: Percentage of children eating vegetables by age category.



Breast feeding was almost universal, 87% of cases and 89% of controls were breast fed (OR 0.64, $p=0.3$). Exposure to cooking fires was significantly more common in controls than in cases in the univariate analysis, the mothers of control children cooked more frequently than the mothers of cases (OR for cooking more than once daily after multivariate adjustment 0.32, 95%CI 0.15, 0.7), but the odds ratios for the other two cooking-fume related variables (the child being carried on the back while cooking and carried on the back while cooking when sick) became non-significant after multivariate adjustment. Maternal asthma was a significant risk factor in the univariate analysis (OR 8, 95% CI: 1, 64, $p=0.05$), but due to the small number of affected mothers ($n=9$), this could not be analysed in the multiple regression analysis.

4.3.1 Discussion

In line with other studies from developing and developed countries, crowding was the most important risk factor for admission to hospital with RSV infection found in our study; ^{19,22,108,117,143,250} more people lived on the compounds of cases than of controls. In the final model, an increasing number of children in the household between the ages of 3 and 5 years was the strongest risk factor identified for admission to hospital with RSV infection. As these children are more mobile than their younger siblings, it is likely that they introduced the virus to the compound, and that more exposure leads to more severe disease as many of the control children are likely to have had mild infection. ⁸⁹ We did not document the number of children older than 5 years of age and of adults on the compound in detail, so we could not explore their influence on severe infection. In contrast, the number of younger children, and the number of children sharing a bed and a room was similar in both groups.

Exposure to cooking fire showed an inverse relation with RSV infection. This is surprising as studies from different developing countries have shown exposure to wood smoke and cooking fires to be a risk factor for ALRI overall, ^{16,38,60} and for bacterial pneumonia in particular. ¹⁸⁹ As the association was consistent in several questions dealing with similar issues, it is unlikely to have been a chance event. It is possible that the relation found in other studies is due to bacterial infections, and that bacterial and viral infections behave differently. Interestingly, a study from Papua New Guinea found a similar inverse relation between obstructive airways disease in children and wood smoke exposure. ⁷ Exposure to tobacco smoke was not related to RSV infection in this study. However, smoking mothers are rare in the Gambia.

In developed countries, breast feeding has been shown to be protective against severe RSV infection.^{44,108,117,226} As most of the children in The Gambia are breast fed up to 18 months of age, it is not surprising that no protective effect of breast feeding could be shown. The only nutritional factor which was more commonly found in controls than in cases was eating vegetables. This was most pronounced in older children. This potentially interesting finding could be explained by the seasonal availability of vegetables. Garden vegetables are generally planted towards the end of the rainy season, and become available in the early dry season. As controls were enrolled on average 3 months later than cases, this association might be caused by the study design. It is possible, however, that part of the seasonality of RSV infection in the Gambia might be explained through the availability of vegetables rather than climatic factors. Similarly, the finding that more mothers of controls were looking after their children during the day in the second study year might be partially explained by seasonal differences of extradomestic work, which might leave the children in the care of another person. One problem of the study was the change of the selection procedure for the controls, which was considered necessary to include more acute parameters, but might have introduced bias into the study. It was therefore comforting to see that all variables had similar odds ratios in both years except the day care by the mother, for which we tried to offer an explanation above.

Genetic factors may play a protective or disease enhancing role. This could explain the finding that the father being non Gambian was more frequent in the cases. The observation that more siblings had died on the case compounds may point in the same direction of a genetic susceptibility to severe disease. However, other differences in life style between groups might explain this finding, for example

mothers who had lost a child previously might be more likely to seek medical attention.

Socio-economic factors did not seem to play a major role in predisposing to severe RSV infection. The housing and environmental conditions in which cases and controls lived were similar, and the educational status of parents of cases and controls did not differ substantially. If socio-economic status played any role, lower social status seemed to be rather more protective. Fathers of controls were more frequently manual workers or policemen, and compounds of cases had more frequently tap water and flush toilets, the association with the latter was strong and independent of other factors in the first model. One possible explanation for this unexpected finding is that better hygiene predisposes to more severe RSV disease. Alternatively, the effect might be explained by differences in care seeking behaviour. Parents of lower socio-economic status might be less willing to present children to hospital even if they have disease of a severity which necessitates admission, or parents of higher status, and mothers who had previously lost a child, might more effectively push their child through the health system to gain admission. For either of these scenarios, children of higher social class would be over-represented in the case group.

Asthma was very uncommon in both cases and controls. However, a history of asthma was obtained significantly more frequently in mothers of cases. This might indicate a small role of atopy in developing severe RSV infection.

In summary, few important differences were found between cases and controls in social and environmental risk factors, despite the fact that the study had at least 80% power to detect an odds ratio of 2 if the variable occurred with a frequency of 10% in the controls. The risk factors found do not explain much of the attributable risk, such

as maternal asthma, are not easily remediable, such as family size and exposure to older children, or considered advances in life style such as flush toilets or taps on the compound. Other factors which are considered to predispose to bacterial pneumonia had an inverse relation with severe RSV infection. Improvements in social and environmental conditions are likely to have little effect, but this requires further studies. For the control of RSV infection in developing countries similar to The Gambia, efforts should concentrate on prevention through vaccination and case management.

4.4 *Sequelae: Cohort study of further respiratory illness*

One hundred and five children with RSV infection were enrolled into a study of the long-term sequelae of severe RSV infection, together with 105 age-matched control children born at the same time as the cases and 102 children born after the respective RSV outbreaks. The age at enrolment (interquartile range) was 8 months (6,11) for cases, 8 months (7,11) for children in control group 1, and 2 months (2,4) for children in control group 2. The number of children ever seen after enrolment either at the clinic or on a follow up visit by a field worker in the case and control groups were 90, 104, and 98, respectively; 76, 67 and 79 respectively, attended MRC at least once for a medical problem. The median time of follow up (interquartile range) was 25 months (22,33) for cases, 25 months (22,32) for control group 1, and 19 months (19,32) for control group 2; the total time of follow up was 2322 months for cases, 2560 months for control group 1 and 2304 months for control group 2. Cases were seen at MRC on 387 occasions, children in control group 1 on 237 occasions, and children in control group 2 on 309 occasions. The median age (interquartile range) of children at the end of the follow up period was 40 (34,42), 39 (34,41) and 33.5 (23,36) months for case and control cohorts 1 and 2, respectively.

The main diagnoses on these occasions are shown in Table 13.

Table 13: Diagnoses during hospital and outpatient attendances of the children in the follow-up cohort.

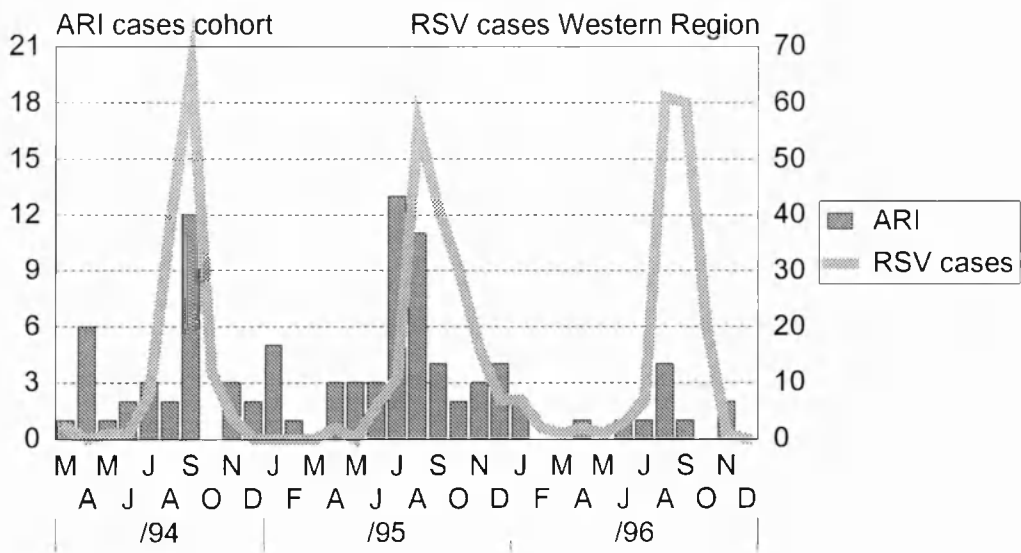
Diagnosis	cases	control 1	control 2
total number of attendances (outpatient and inpatient)	387	237	309
pneumonia	83	34	37
ALRI with wheezing	22	7	7
otitis media	16	4	11
gastro-enteritis	57	48	70
bloody diarrhoea	10	3	7
mouth infection	8	2	6
skin infection	57	36	46
malaria	39	23	33

Note: Multiple diagnoses could be made.

Children from the 3 groups were admitted to hospital for inpatient treatment on 47, 33, and 40 occasions, respectively. Pneumonia was diagnosed on at least one occasion in 28 case children (31% of those ever seen), 12 (12%) children in control cohort 1, and 14 (14%) children in control cohort 2. Wheezing was diagnosed in 13 (14%), 2 (2%), and 5 (5%) children in the three cohorts, respectively. Most of the episodes of respiratory illness were seen during the months from July to December (Fig. 25).

Ten children had proven episodes of RSV infection: 4 in the case group, one in control group 1, and 5 in control group 2. One, 1, and 4 children in these groups were admitted for inpatient care, respectively. Four of the episodes were in 1994, and 6 in 1995.

Figure 25: Number of children diagnosed as having pneumonia or wheezing in the follow up cohorts (ARI cases cohort), compared with the seasonality of RSV infection in the Western Region of The Gambia as determined by hospital surveillance (RSV cases).



Using Poisson regression to determine a model predicting pneumonia, wheezing, pneumonia or wheezing, and admission with pneumonia or wheezing, being a child in the case cohort, age, season, and care seeking behaviour were found to be significant predictors, after adjustment for the other variables, for pneumonia and pneumonia or wheezing, being a case, season and age for wheezing, and being a case, age and care seeking behaviour for being admitted with pneumonia or wheezing. Sex and being a child in control cohort 1 or 2 were not significant. Incidence rate ratios between cases and the combined control groups are shown in Table 14.

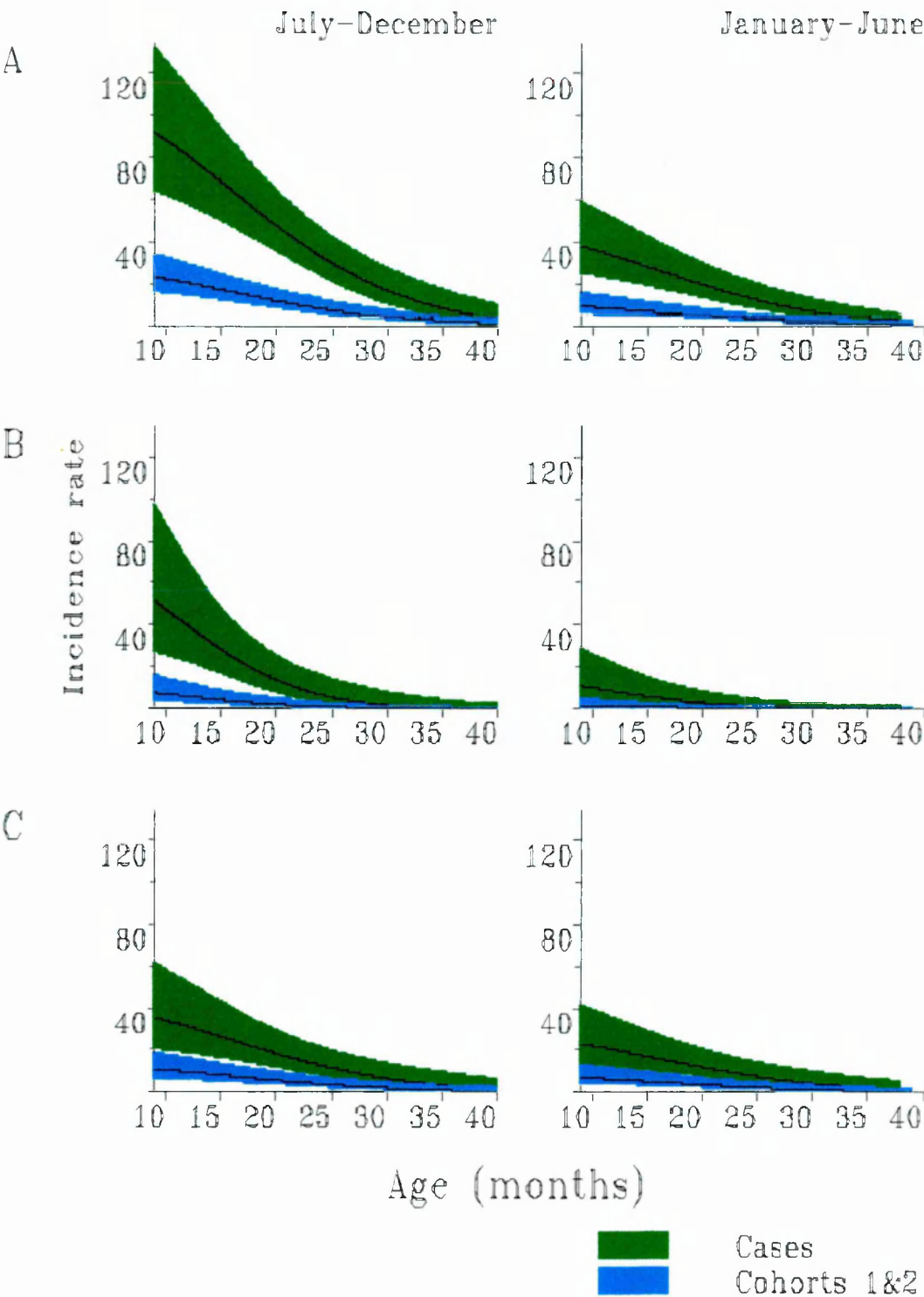
Incidence rates were higher for younger children, and more than twofold higher during the second half of the year than during the first (Figure 26).

Table 14: Adjusted incidence rate ratios (IRR) for pneumonia, wheezing, pneumonia or wheezing, and pneumonia or wheezing admitted and for being a case, seasonality, age, care seeking behaviour, and sex by Poisson regression analysis.

Variable	Categories	Pneumonia		Wheezing		Pneumonia or wheezing		Pneumonia or wheezing, admitted	
		IRR	[95% CI]	IRR	[95% CI]	IRR	[95% CI]	IRR	[95% CI]
Case	vs. controls	3.80	[2.37, 6.10]	7.33	[3.10, 17.54]	3.96	[2.60, 6.04]	3.40	[1.87, 6.15]
Season	Rainy vs. dry	2.09	[1.29, 3.38]	4.90	[1.82, 13.19]	2.43	[1.56, 3.77]	1.53	[0.85, 2.75]
Age	Per year	0.46	[0.33, 0.65]	0.20	[0.096, 0.41]	0.40	[0.30, 0.55]	0.41	[0.26, 0.63]
Care seeking behaviour	More than one attendance other than for ARI	2.62	[1.52, 4.51]	1.15	[0.34, 3.84]	2.34	[1.43, 3.84]	2.45	[1.22, 4.95]
Sex	Male vs. female	0.79	[0.50, 1.25]	1.16	[0.52, 2.59]	0.94	[0.63, 1.41]	0.62	[0.35, 1.11]

Figure 1: Incidence rates (95% CI) of pneumonia (A), wheezing (B), and admission for pneumonia or wheezing (C) by age and season.

The two control cohorts were combined, they were not significantly different from each other after adjustment.



Derived from the model, the point estimates of the incidence rate/100 child years for pneumonia in the dry season (January to June) for 12 months old children were 27 for cases, 8.1 for control cohort 1 and 6.5 for control cohort 2. For wheezing, the equivalent estimates were 8.4, 0.9, 1.4, respectively. At 3 years of age, the incidence rates had fallen to 3.5, 1.1, 0.9 and 0.1, 0.01, 0.01 for the three groups and for pneumonia and wheezing, respectively.

The nutritional status of children compared to the NCHS standard varied with age, dropping below the standard above half a year of age. However, it was not significantly different between the groups.

During the follow up period, 5 children died (1 case, 3 control cohort 1 children, and 1 control cohort 2 child).

4.4.1 Discussion

Several studies from developed countries have shown an increased frequency of wheeze in children after severe RSV infection.^{45,113,132,139,142,154,202,204,225} Similarly, a study from Qatar showed recurrent wheezing in 42 % of children after RSV infection.¹⁹⁵ We have found that wheezing is also significantly more common in Gambian children after RSV infection than in controls, but the striking finding in our study was the high number of lower respiratory infections without wheeze in cases. These were 3 - 4 fold more common in cases than in the control cohorts. In addition, all respiratory infections were approximately two times more frequent during the second half of the year, which corresponded to the time of RSV outbreaks during the study years. Thus, it is possible that about half of the respiratory infections during that time, whether with wheeze or without, were

caused by RSV. A third striking finding from our study is the rapid decline in the incidence of respiratory infections with age. Several explanations have been brought forward in the past to explain the higher incidence of wheezing after RSV infection. They include the suggestion that RSV damages airways and therefore leaves children more vulnerable,¹⁵⁷ that RSV induces an atypical immune response with an imbalance of Th1 and Th2 cells and therefore induces asthma,^{21,194,213} that children with atopy are selected by RSV and therefore continue to wheeze,¹⁴⁵ or that RSV affects children with smaller airways most severely and that RSV only identifies this at-risk group, which continues to wheeze.^{154,273} We cannot decide from our study whether RSV was causing damage to the airways which led to further infections, or whether RSV infection was merely an indicator of an abnormal lung which would have been infected more frequently afterwards anyhow. However, the rapid decline of the incidence of all respiratory infections with age indicates that the responsible factor loses its influence with increasing age, as would be expected if small airways were the responsible factor or if children acquire immunity protecting them, rather than an abnormal priming such as atopy or an abnormal Th1/Th2 ratio. The latter would be expected to continue unabated or even increase with age.

One of the confusing points in the discussion of the interrelationship between RSV infection, asthma and wheezy bronchitis is their identical clinical presentation. Wheezy children have a variety of underlying causes. In The Gambia, clinically apparent asthma is relatively rare. In the parallel risk factor study, only 4% of mothers of RSV children said they had asthma (see chapter 4.3). This might explain the rapid disappearance of wheezing episodes as a

clinical problem in all groups, as the development of asthma, based on atopy and independent of RSV, does not play as big a role as in industrialised countries.

Our estimates are based on passive case finding, as the guardian had to bring the child to MRC for assessment and treatment. The estimates presented are therefore likely to be underestimates. It is unlikely, however, that we missed many of the more severe events, as free medical care from a doctor at MRC was an attractive incentive for parents. Children from the case cohort presented more frequently for any condition. This could indicate a higher susceptibility to any disease in this group, or, more likely, a difference in care seeking behaviour, which we have adjusted for in the analysis.

In summary, Gambian children with RSV infection continue to have a higher number of respiratory problems than controls over the next 2 years of life. The frequency of respiratory problems declines with age, however, and the overall long term prognosis of children after RSV infection in The Gambia appears good.

Chapter 5. Summary and conclusions

5.1 Findings

In the work presented in this thesis, I have explored the importance of RSV infection in The Gambia, West Africa from 1993 to 1996. RSV was sought in children under the age of two years admitted to three hospitals in the Western Region of The Gambia by immunofluorescence of nasopharyngeal aspirate samples. Routine records of all children with ALRI were analysed, and the incidence rates of all ALRI, severe RSV infection, and hypoxaemic RSV infection were compared.

In a regression model adjusting for size of village and rural or urban environment, the incidence rates of ALRI leading to hospital admission among children < 1 year of age living near to the hospitals were estimated to be 11.4 (95%CI 10.6, 12.3) per hundred children per year for boys and 9.1 (8.4, 9.9) for girls living in urban areas. In rural areas incidence rates were 16.1 (14.6, 17.6) for boys and 12.8 (11.6, 14.1) for girls. For severe RSV infection, the incidence was 1.3 (1.1, 1.6) per hundred children per year for boys, and 1.0 (0.8, 1.3) for girls, in both urban and rural areas. For hypoxaemic RSV infection, the corresponding estimates were 0.1 (0.07, 0.19) and 0.07 (0.04, 0.13) for boys and girls respectively. Correcting for omissions in sampling, the annual incidence of ALRI cases admitted to hospital which were due to RSV was estimated to be 2.6% for boys and 2% for girls; the proportion of all ALRI admissions due to RSV was 19%. The median ages of children seen in 1993 to 1996 were 3, 7, 8,

and 5 months, respectively. Sixty-two percent of children less than 6 months of age were boys. Thirteen children (2.4%) had conditions considered to increase the risk of severe RSV infection. Eighty (16%) children received oxygen because of hypoxaemia. Nine bacterial cultures (7 of 255 blood cultures [2.7%]) were positive. Thirteen children died. During the 4 study years, 90%, 25%, 75% and 95% of isolates typed were RSV subgroup A.

In a community study, 25% of children living in compounds of cases and 31% of those living in the compounds of controls had evidence of RSV infection during the period of surveillance.

Possible risk factors for severe RSV infection were investigated in a case-control study. Two hundred and seventy-seven children admitted to three hospitals in the Western Region of the Gambia with an ALRI due to RSV were compared with 364 control children matched for age and location of residence who had not been admitted to hospital with an ALRI during the RSV season. A detailed questionnaire covering a wide range of potential social, environmental and nutritional risk factors was administered to the child's guardian. Cases came from larger or more crowded compounds than controls; increased risk was associated particularly with greater numbers of children in the age group 3-5 years living in the compound (odds ratio (OR) for 2 or more children in the age group 3 to 5 years: 5.67, 95% CI: 2.8, 11.5). Cases had a sibling who had died more frequently than controls (OR 2.72, (95%CI 1.4, 5.28). Controls were more likely to have been exposed to smoke from cooking fires than cases (OR for the mother of cases cooking at least once daily 0.32, 95%CI: 0.15, 0.7). Other

protective factors were father's nationality and some professions. Vegetables were included in the diet of controls more frequently than in that of cases (OR 0.18, 95%CI: 0.07, 0.48). Mothers of cases complained of asthma more frequently than mothers of controls, but the number of asthmatic mothers was small (4.2 vs. 0.5%, $p=0.05$).

To obtain information about the long term respiratory sequelae of severe RSV infection, we formed a cohort of 105 children admitted to hospital with RSV infection (cases), 105 control children matched for age who had not been admitted to hospital with ALRI during the previous RSV season (cohort 1) and 102 controls who had been born after the RSV season (cohort 2), and followed them prospectively for over 2 years through passive, clinic-based surveillance with free access to a physician. The frequencies of pneumonia, wheezing, and admission with ALRI were analysed. Using Poisson regression to determine a model predicting pneumonia or wheezing, and after adjusting for age, season and care seeking behaviour, the incidence rate ratio (95%CI) for pneumonia was 3.80 (2.73, 6.10), comparing cases with both control cohorts combined. For wheezing, the rate ratio was 7.33 (3.10, 17.54), for pneumonia or wheezing 3.96 (2.60, 6.04), and for admission with pneumonia or wheezing 3.40 (1.87, 6.15). The point estimates of the incidence rate/100 child years for pneumonia in the dry season for 12 month-old children were 27 for cases, 8.1 for control cohort 1 and 6.51 for control cohort 2. For wheezing, the same estimates were 8.38, 0.88, 1.41, respectively. At 3 years of age, the incidence rates had fallen to 3.52, 1.05, 0.8 and 0.08, 0.01, 0.01 for the three groups and pneumonia and wheezing,

respectively. Incidence rates were approximately twofold higher in the wet season.

This series of studies has shown that RSV is a significant cause of lower respiratory tract infection in young children in The Gambia, causing yearly epidemics of ALRI. It poses a significant burden on the health system, especially through the demand for supplementary oxygen. The clinical spectrum of RSV infection in The Gambia is similar to that seen in developed countries. Addressing potential risk factors is unlikely to be a feasible strategy in the control of RSV in The Gambia. Pneumonia and wheezing are significantly more common in children after RSV infection than in controls; the incidence is higher in the rainy season, and declines rapidly with increasing age.

5.2 Outlook

It was not possible in these studies to assess the importance of RSV as a cause of mortality in young children in The Gambia. By the time that a child has been diagnosed as having RSV in a hospital, he has overcome the worst risk of dying. The question remains as to how many children die before they are admitted to hospital. As the RSV season coincides with the malaria season, and because both infections can present with similar signs and symptoms, a randomised vaccine trial with an effective vaccine is probably the only way in which this issue could be resolved. We have shown that even if RSV is not a major cause of death in infancy, morbidity from the infection is considerable, risk factors are not easily remediable, and children who have had a severe RSV infection tend to

have more respiratory problems in the next few years. Thus, on these grounds alone a vaccine would be likely to be cost effective in The Gambia and in other developing countries where the pattern of RSV infection is similar.

During the period of the study, RSV outbreaks occurred regularly each rainy season. However, in 1997, a small outbreak occurred after the end of the rainy season. The timing of outbreaks of RSV infection appeared to be erratic in earlier years also. Knowledge of the seasonality of RSV outbreaks is important in the planning of a vaccine trial so that intensive surveillance is instituted during the optimum period. In addition, information on seasonality is useful for the mathematical modelling of disease dynamics and thus for understanding patterns of transmission.

Short of a randomised RSV vaccine trial, documentation of cases of death of children in the community and the correlation of deaths with documented RSV activity in the same community would provide some estimate of attributable mortality, if the seasonality of RSV infection differs in between years from that of malaria, as it was the case in 1997. A large, rural vaccine trial such as the proposed trial with a vaccine against *S. pneumoniae* could provide a vehicle for that, if surveillance for RSV was added.

We obtained information on the incidence of respiratory infections in children who had experienced a clinically important RSV infection and in controls during a period of up to 3 years after their infection (Chapter 4.4). It would be of

interest to continue follow-up of these children to an age when tests of lung function, such as measurement of the size of bronchial airways and the state of bronchial responsiveness, could be more readily undertaken and when further investigations such as skin tests, tests of T cell function and DNA studies for markers of atopy could be done.

If, during these further follow-up studies, abnormal lung function was found more frequently in case than in control children, the question will still remain open as to whether this was due to a pre-existing abnormality before the RSV infection, or whether the case children acquired the abnormalities as a result of their RSV infection. This problem could only be resolved definitively by recruiting a large cohort of infants at birth, performing a lung function test suitable for babies shortly after birth on all children and following them longitudinally to determine if they developed an RSV infection and, if so, its severity.

A prospective community cohort study would also be desirable to obtain more information on the spread of infection in the community, information that would be helpful in the planning of a vaccine trial. Ideally, a large number of compounds would be recruited and visited at least weekly by a field worker, who would document new episodes of respiratory infection and obtain a nasopharyngeal aspirate sample when this was reported. This would ensure that the beginning of an outbreak was not missed. As the sampling does not depend on an index child, who might be the last to be infected, reliable estimates on the

number of infected children would be obtained, and compounds with children with severe infection could be compared with those with mild cases. Serum samples obtained at intervals would improve the precision of the estimate, as seroconversion could be used in addition to antigen detection.

The Gambia would be a suitable site for some of these further studies, as so much information on RSV is available already. However, to improve the global view of the importance of RSV, a number of other developing countries or agencies interested in child health in these countries should be encouraged to undertake more in depth studies on RSV. It is encouraging to know that the Global Programme for Vaccines of the World Health Organisation is currently supporting studies on RSV epidemiology in several developing countries, providing information which should contribute to the knowledge base about this virus world-wide. Ultimately, it should be possible to control severe respiratory disease due to RSV through an effective vaccine.

Chapter 6. References

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